

## INHIBITORS OF THE MAP KINASE PATHWAY

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### Field of the Invention

This invention relates to the development and use of human therapeutics that inhibit intracellular signaling via the MAP kinase pathways.

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### Background of the Invention

The evolutionarily conserved Ras-MAPK signaling network regulates diverse biological processes such as cell proliferation, differentiation, migration, and survival. Many of the regulators and effectors within this network have been implicated in diverse pathological processes. MAP kinases and their targets have  
15 been identified as, for example, potent oncogenes or tumor suppressor genes and proinflammatory mediators.

Normally, the MAPK network is activated when growth factors or hormones bind to cell surface receptors. The extracellular signal is amplified and converted into an appropriate biological response. However, dysfunction of any  
20 component of the signaling pathway may result in a pathological condition. Cancer, for example, is a disease marked by the uncontrolled growth of abnormal cells. Cancerous cells have overcome the barriers imposed in normal cells, which have a finite lifespan, and grow indefinitely. As the growth of cancer cells continues, genetic alterations can accrue and persist so that the cancerous cell  
25 displays increasingly aggressive growth phenotypes. If left untreated, metastasis, the spread of cancer cells to distant areas of the body by way of the lymph system or bloodstream, may ensue, destroying healthy tissue and, ultimately, leading to death.

According to a recent American Cancer Society study, at least 1,268,000 new cancer cases are expected to be diagnosed in the United States in any given year.

However in cancer cells, mutations in upstream activators of MAPK, such as Ras or Raf, lead to constitutive signaling even in the absence of growth factors.

5 Constitutively activating mutations in Ras are detected in at least 30% of all human malignancies but are present in especially high levels in colon (50%) and pancreatic cancers (90%). The activation kinetics of the ERK1/2-MAPK signaling pathway have also been associated with distinct biological outcomes. In fibroblasts, sustained ERK1/2 activation over several hours induces entry into S  
10 phase of the cell cycle while transient (20-30 min) activation does not.

In various cell types, the ERK1/2 pathway also has a critical role in regulation of nucleotide biosynthesis, transcription, migration, cell survival, differentiation and adaptive responses. Specifically, ERK signaling can control cardiomyocyte cell growth and the response to ventricular heart failure, cell  
15 survival in atherosclerosis, various metabolic processes such as glucose uptake, protein synthesis and leptin signaling, regulation of the immune response such as in T cell activation and inflammatory cytokine signaling, and mediating the effect of neurotransmitters that control memory and behavior. ERK signaling also can control the induction of genes that are required for establishing circadian rhythms.

20 Accordingly, small molecule drugs that can selectively inhibit regulatory proteins within the ERK1/2-MAPK pathway have enormous therapeutic potential.

General MAPK inhibitors, however, are likely to be toxic due to the many metabolic and proliferative functions regulated by this pathway in healthy cells. ERK1/2 specifically recognizes some physiological substrates through the  
25 presence of ERK1/2 docking sites in substrates (Jacobs *et al.*, 1999; Tanoue *et al.*, 2000). At least two classes of docking site have been identified and are known as the D-box and DEF domain.

Substrate docking directs ERK1/2 to phosphorylate specific amino acids known to regulate the biological function of the substrate. Interaction of ERK1/2 with the D-box docking site is required for ERK's initial activation by MEK, as well as its inactivation by phosphatases (Tanoue *et al.*, 2000). By contrast, the  
5 DEF domain appears to be mainly found in downstream targets of ERK1/2 (Jacobs *et al.*, 1999).

### Summary of the Invention

We have discovered that MAP kinases (e.g., extracellular signal-regulated  
10 kinase 1/2 (ERK1/2)), bind to certain target proteins (e.g., immediate early gene (IEG) products) through a DEF domain. This specific binding interaction results in the phosphorylation of target residues and a resulting biological effect (e.g., progression through the cell cycle). Blocking the binding events we have  
15 identified allows treatment of a variety of human diseases where the interaction of MAP kinases with the DEF domain of the target proteins has a causative biological effect.

Accordingly, in one aspect, the present invention provides for a method of identifying therapeutic compounds that affect the MAP kinase-DEF domain interaction. The method consists of the steps of: (i) providing test cells that  
20 express a target protein having a DEF domain and a MAP kinase, and are capable of progressing through the cell cycle; (ii) culturing the test cells in the presence of a growth factor, cytokine, tumor promoter, or oncogene under conditions that activate the MAP kinase; (iii) contacting the test cells with the candidate  
25 compound; (iv) assessing the binding of said MAP kinase to the DEF domain of the target protein relative to the binding in the absence of said candidate compound, wherein a candidate compound that inhibits the binding is identified as a therapeutic compound. Desirably, the test cells are mammalian; most desirably

human. Suitable test cells include, for example, a primary cell line, an immortalized cell line, or a tumor cell line. Fibroblasts (e.g., NIH 3T3 cells) are particularly useful test cells, but any mammalian cell type can be used because IEGs are ubiquitously expressed. Useful growth factors, cytokines, tumor promoters, and oncogenes include, for example, epidermal growth factor (EGF) and EGF-related factors including, for example, transforming growth factor  $\alpha$  (TGF $\alpha$ ), heparin-binding-like EGF, heregulin, amphiregulin, epiregulin, cripto, platelet derived growth factor (PDGF), including PGDF-AA, PGDF-BB, and PGDF -CC, insulin, insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), colony stimulating factor (CSF), and hepatocyte growth factor (HGF). Useful cytokines include, for example, the chemokines, interleukins, and lysophosphatidic acid (LPA). Useful tumor promoters include, for example, phorbol esters, phosphatase inhibitors such as okadaic acid, microcystin, vanadate, hydrogen peroxide, and calyculin A. Useful oncogenes include, for example, Erb2/neu, sis, kit, Ras, Raf, PI3-kinase, and PTEN. Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) are particularly useful growth factors. In other embodiments in which the target protein is c-Fos, the binding of the MAP kinase to c-Fos is assessed by measuring the phosphorylation of T325 or T331. Preferably, this is performed using a phospho-T325-specific antibody.

In another aspect, the invention provides a method for identifying a therapeutic compound by (i) providing a sample that contains a polypeptide having a DEF domain, a MAP kinase, and a candidate compound, (ii) contacting the target protein, the MAP kinase, and the candidate compound, and (iii) assessing the binding of the MAP kinase to the DEF domain of the target protein in the sample in the presence of the candidate compound relative to binding in the absence of the candidate compound, wherein a compound that inhibits binding of the MAP kinase to the target protein is identified as a therapeutic compound. In desirable

embodiments, the target protein further contains a fluorescent moiety (e.g., fluorescein).

In preferred embodiments of the previous two aspects, the MAP kinase is ERK1/2. In other desirable embodiments, the target proteins are members of the Fos, Jun, and Myc family proteins. Specifically, desirable target proteins include c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, c-Jun, in addition to Egr-1 and mPer1. In one embodiment, the target protein contains the sequence of a protein identified in Table 1 or 2 and the identified therapeutic is useful for treating cancer. In another embodiment, the target protein contains the sequence of a protein identified in Table 3 and the identified therapeutic is useful for treating a cardiovascular disease. In another embodiment, the target protein contains the sequence of a protein identified in Table 4 and the identified therapeutic is useful for treating an inflammatory disorder. In another embodiment, the target protein contains the sequence of a protein identified in Table 5 and the identified therapeutic is useful for treating a metabolic disorder. In another embodiment, the target protein contains the sequence of a protein identified in Table 6 and the identified therapeutic is useful for treating a neuropathy or a behavioral disorder. In another embodiment, the target protein contains the sequence of a protein identified in Table 7 and the identified therapeutic is useful for treating a sleep disorder. In other embodiments, the target protein contains a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1). Assessment of target residue phosphorylation is desirably performed using a phospho-specific antibody.

In another aspect, the invention provides a method for treating cancer in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target

protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is a member of the Fos, Jun, and Myc family proteins including, for example, c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, and c-Jun. Alternatively, the target protein is one identified in Tables 1 or 2. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a cardiovascular disease in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 3. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating an inflammatory disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 4. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a metabolic disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 5. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a neuropathy or a behavioral disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 6. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

In another aspect, the invention provides a method for treating a sleep disorder in a mammal (e.g., human) by administering a therapeutically effective amount of a compound that inhibits the binding of a MAP kinase to the DEF domain of a target protein. Preferably, the MAP kinase is ERK 1/2, and the target protein is one identified in Table 7. Alternatively, the compound inhibits the binding of a MAP kinase to a DEF domain having the amino acid sequence F/Y-X-F/Y-X (SEQ ID NO: 28), preferably the sequence is F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1).

Particularly useful DEF domain inhibitors for any of the therapeutic methods are polypeptides having the sequence F/Y—X—F/Y—X (SEQ ID NO: 28; “naked DEF domains”) and chimeric proteins that contain a DEF domain inserted into a non-target protein. In preferred embodiments, the DEF domain has the sequence F/Y-X-F/Y-P (SEQ ID NO: 29), more preferably the sequence is F-X-F-P (SEQ ID NO: 1). The most desirable chimeric proteins are based on non-target proteins that affect the pharmacokinetic or pharmacodynamic properties compared to administering the naked DEF domain alone. For example, DEF domains may be incorporated into serum albumin or cereloplasmin.

10 The compound is administered in an amount, frequency, and duration that is therapeutically effective for treating the diagnosed condition. Desirably, the compound is administered in an amount between 0.01 and 3000 mg/day, more preferably, in an amount between 0.1 and 2000 mg/day, either orally or by injection (i.e., intravenous, intramuscular, or subcutaneous). Alternatively, the  
15 compound can be administered as a 0.5% to 25% topical formulation.

Therapy may be provided in any appropriate location: at home, the doctor’s office, a clinic, a hospital’s outpatient department, or a hospital. Treatment generally begins at a hospital so that the doctor can observe the therapy’s effects closely and make any adjustments that are needed. The duration of the therapy  
20 depends on the condition being treated, the age and condition of the patient, the stage and type of the patient’s disease, and how the patient’s body responds to the treatment. Drug administration may be performed at different intervals (e.g., daily, weekly, or monthly).

In another aspect, the invention provides an antibody that specifically binds  
25 to phospho-T-325 c-Fos. The antibody may be monoclonal or polyclonal.

In another aspect, the invention provides a pharmaceutical formulation that contains a therapeutic compound identified by either of the first two aspects of the invention, and a pharmaceutically acceptable carrier. The pharmaceutical formulation may be suitable for oral administration, injection, or topical application.

By “specifically binds to phospho-T-325 c-Fos,” when referring to the antibodies of this invention, is meant an antibody that binds with high affinity ( $<10^{-8}\text{M}$ ) to native c-Fos in which the threonine at amino acid position 325 is phosphorylated, but does not significantly bind to c-Fos in which the T325 is unphosphorylated. Desirably, the difference in specificity of antibody binding between phospho-T-325 c-Fos and the unphosphorylated form is at least 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, or 1000-fold.

By “DEF domain” is meant a polypeptide having the amino acid sequence: F/Y—X<sub>1</sub>—F/Y—X<sub>2</sub> (SEQ ID NO: 28), wherein F is phenylalanine, Y is tyrosine, P is proline, and X<sub>1</sub> and X<sub>2</sub> are any naturally-occurring or non-naturally-occurring amino acids. Desirably, X<sub>2</sub> is proline.

By “target protein” is meant any protein that contains a DEF domain capable of binding a target kinase (e.g., a MAP kinase). Desirable target proteins are phosphorylated by the MAP kinase ERK1/2 following ERK1/2 binding to the DEF domain. Target proteins include, for example, gene products of the immediate early genes from the Fos, Myc, and Jun families, proteins identified in Tables 1-7, or chimeric or synthetic proteins into which a DEF domain has been inserted by artifice. Specific target proteins include, for example, c-Fos, Fra-1, Fra-2, cMyc, N-Myc, JunD, JunB, c-Jun, Egr-1, and mPer1.

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By “target residue(s)” is meant one or more residues of a target protein that are N-terminal to the DEF domain and that are phosphorylated as a result of the binding of a MAP kinase. Target residues include, for example, T325 and T331 of c-Fos. This phosphorylation event is also termed a “DEF domain-dependent phosphorylation.”

By “primed,” when referring to a target protein, is meant a phosphorylation event that makes a DEF domain available for binding of a MAP kinase. Thus, the amino acid residues that are the subject of a “priming” modification are not the same as the target residues. For example, c-Fos is primed when S362 and/or S374 are phosphorylated or substituted for aspartate or glutamate.

By “target kinase” is meant a protein kinase that is capable of binding a DEF domain and phosphorylating a target residue. Target kinases include the MAP kinases such as ERK1/2, for example. Thus, an “activated target kinase” is one that itself has undergone a post-translational modification causing an increase in kinase activity and/or inducing a change in subcellular localization. For example, in order to be fully activated and translocated from the cytoplasm to the nucleus, ERK1/2 is phosphorylated.

By “DEF domain inhibitor” is meant any chemical compound (i.e., polypeptide or non-peptide) that inhibits the interaction of a target kinase (i.e., ERK1/2 or RSK) with the DEF domain of a target protein.

The term “assessing the binding of a MAP kinase to a DEF domain,” is meant to include any appropriate binding or biochemical assessment which may be either qualitative or quantitative. This term specifically includes, for example, directly assessing the interaction of the MAP kinase and the DEF domain. Alternatively, assays that measure biochemical outcomes of a MAP kinase – DEF domain binding event are useful. These assays include, for example, measuring the amount of DEF domain-dependent phosphorylation.

As exemplified in detail below, the phosphorylation of T325 and/or T331 of c-fos is dependent upon this binding event.

By “cancer” is meant neoplastic cells multiplying in an abnormal manner. In a cancer, growth is uncontrolled and progressive, and occurs under conditions  
5 that would not elicit, or would halt the multiplication of non-cancerous cells. Cancer includes, for example, leukemias and lymphomas (Hodgkin’s disease, non-Hodgkin’s disease), as well as solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, liposarcoma, osteogenic sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma,  
10 basal cell carcinoma, adenocarcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, Wilm’s tumor, cervical cancer, uterine cancer, testicular cancer, small and/or non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

By “treating cancer” is meant a therapy that measurably slows, stops, or  
15 reverses the growth rate of the cancer (i.e., neoplastic cells) *in vivo*. Desirably, a slowing of the growth rate is by at least 20%, 30%, 50%, or even 70%, as determined using a suitable assay for determination of cell growth rates (e.g., a cell growth assay described herein). Typically, a reversal of growth rate is  
20 accomplished by initiating or accelerating necrotic or apoptotic mechanisms of cell death in the neoplastic cells, resulting in a shrinkage of the neoplasm. Efficacy of a treatment may be measured by any means known to those skilled in the art including tumor imaging or measurement of neoplastic markers.

By “cardiovascular disease” is meant ischemic heart disease, ventricular  
25 heart failure, cardiac hypertrophy, hypertension, and atherosclerosis.

By "inflammatory disorder" is meant any condition that is characterized by inflammation as a primary or secondary symptom. Inflammatory disorders include, for example, allergic or autoimmune disorders, anaphylaxis, and septic shock. Examples of allergic disorders include allergic rhinitis, asthma, atopic dermatitis, and food allergies. Examples of autoimmune disorders include, but are not limited to, type 1 insulin-dependent diabetes mellitus, inflammatory bowel disease, Crohn's disease, ulcerative colitis, dermatitis, meningitis, thrombotic thrombocytopenic purpura, Sjögren's syndrome, encephalitis, uveitis, leukocyte adhesion deficiency, rheumatoid and other forms of immune arthritis, rheumatic fever, Reiter's syndrome, psoriatic arthritis, progressive systemic sclerosis, primary biliary cirrhosis, pemphigus, pemphigoid, necrotizing vasculitis, myasthenia gravis, multiple sclerosis, lupus erythematosus, polymyositis, sarcoidosis, granulomatosis, vasculitis, pernicious anemia, CNS inflammatory disorder, antigen-antibody complex mediated diseases, autoimmune hemolytic anemia, Hashimoto's thyroiditis, Graves disease, habitual spontaneous abortions, Reynard's syndrome, glomerulonephritis, dermatomyositis, chronic active hepatitis, celiac disease, autoimmune complications of AIDS, atrophic gastritis, ankylosing spondylitis and Addison's disease.

By "metabolic disorder" is meant a disease that interferes with the normal metabolic function of cells, tissues or organs. Metabolic disorders include, for example, diabetes, obesity, jaundice, polycystic kidney and hepatic disease, pancreatitis, Graves' disease, and Werner's syndrome. Metabolic diseases may also arise as secondary complications of another disease such as one involving a tumor. For example, cachexia or muscle wasting, and metabolic and digestive complications often arise from the presence of pancreatic, colonic, stomach, hepatic and hepatocellular tumors.

By "neuropathy" is meant any condition of the central or peripheral nervous system characterized by axonal loss that may or may not be accompanied by neuronal loss. Neuropathies specifically include conditions affecting sensory and motor neurons and include, for example, diabetic neuropathy, muscular dystrophy, Williams Beuren's Syndrome.

By "behavioral disorder" is meant any condition affecting motivation, emotion, learning, or memory. Behavioral disorders are also meant to broadly encompass neurodegenerative diseases. Thus, behavioral disorders include, for example, psychosis, schizophrenia, autism, Down's Syndrome, Parkinson's Disease, Alzheimer's Disease, epilepsy, Cockayne syndrome, bipolar disorders, and depression. Also included are addictions including, for example, addictions to opiates and barbiturates.

By "sleep disorder" is meant any condition that primarily affects sleep and consciousness. Sleep disorders include, for example, advanced sleep phase syndrome, delayed sleep phase syndrome, insomnia and narcolepsy.

By "a therapeutically effective amount" is meant the amount of a compound required to treat cancer (i.e., inhibit the growth of the neoplastic cells). The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of neoplasms (i.e., cancer) varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen.

### Brief Description of Drawings

FIGURE 1 is a series of photomicrographs showing the differential responsiveness of Swiss 3T3 fibroblasts to growth factors. FIGURE 1A shows quiescent Swiss 3T3 cells (-) that were treated with EGF (25 ng/ml) or PDGF (20

ng/ml) for 20 h and then processed for BrdU incorporation, as described below. FIGURE 1B shows quiescent Swiss 3T3 cells that were treated with PDGF or EGF for the indicated times and ERK1/2 and RSK kinase activities were determined using immunocomplex kinase assays. The fold activation at each time is indicated above each lane. FIGURE 1C is the indirect immunofluorescence detection of hyperphosphorylated activated ERK1/2 in Swiss 3T3 cells treated with EGF or PDGF. FIGURE 1D is the indirect immunofluorescence detection of c-Fos in Swiss 3T3 cells treated with EGF or PDGF.

FIGURE 2A is an illustration showing the residues in c-Fos that are phosphorylated by RSK and ERK1/2 *in vivo*. FIGURE 2B shows the electrophoretic separation of cell extracts from parallel cultures of 208F fibroblasts stably expressing Fos-WT (WT), Fos-AA (AA) or Fos-DD (DD) that were metabolically labelled with <sup>35</sup>S-methionine or <sup>32</sup>P-orthophosphate and cultured with or without 10% FBS for 15 min. Fos proteins were immunoprecipitated from cell lysates and analysed by SDS-PAGE. The serum-stimulated phosphorylation of Fos-DD was consistently two to threefold greater than Fos-AA and arrows indicate the major mobilities observed after stimulation. FIGURE 2C is Western blot from NIH 3T3 cells transfected with Fos-WT (WT), Fos-AA (AA) or Fos-DD (DD) were serum-starved and then pre-treated with 5  $\mu$ M UO126 (+) or 0.1% DMSO (-) for 30 min before treatment with EGF (+) for 5 min. FIGURE 2D is an autoradiogram of an *in vitro* phosphorylation of the indicated (His)<sub>6</sub>-Fos proteins by endogenous ERK1/2 from quiescent or EGF-stimulated NIH 3T3 cells. Results shown are representative of three independent experiments. Fos-EE (S362E/S374E) was used as primed c-Fos for *in vitro* phosphorylation studies.

FIGURE 3A is an illustration that details the DEF domain at the C-terminus of c-Fos. The phosphorylation of Fos-EE in the absence of peptide competitor is expressed as 100%. *In vitro* phosphorylation of (His)<sub>6</sub>-Fos-EE was performed as

described below. The data shown are the means  $\pm$ SEM from three experiments. FIGURE 3B is a graph showing the inhibition of Fos-EE phosphorylation by peptides containing a DEF domain (FQFP; SEQ ID NO: 3) or a mutated DEF domain (AQAP; SEQ ID NO: 4). FIGURE 3C is a graph showing the inhibition of Fos-EE phosphorylation by peptides containing the c-Fos DEF domain (FTYP; SEQ ID NO: 2) or a mutated DEF (ATYP; SEQ ID NO: 5). FIGURE 3D shows the results of a Western blot of NIH 3T3 cells transfected with the indicated FLAG-Fos-DD (DD) alleles were left quiescent (-) or were stimulated (+) with EGF for 5 min before lysis. Arrows indicate the major Fos-DD mobilities. FIGURE 3E is a Western blot of cells transfected with the indicated FLAG-Fos alleles. Arrows show the three major Fos-WT mobilities associated with growth factor stimulation.

FIGURE 4A is an illustration identifying the ERK1/2 phosphorylation sites N-terminal to the DEF domain in c-Fos. *In vitro* phosphorylation of (His)<sub>6</sub>-Fos-EE proteins by activated (His)<sub>6</sub>-ERK1/2 was performed. The phosphorylation of the Fos-EE point mutants is expressed as a percentage of Fos-EE (100%). The data shown are the means  $\pm$ SEM from three experiments. FIGURE 4B is a Western blot of NIH 3T3 cells that were transfected with the indicated FLAG-Fos-DD (DD) alleles. Cells were treated with EGF for 5 min or left untreated. FIGURE 4C is a Western blot of cells transfected with the indicated FLAG-Fos alleles and treated as in Figure 3B.

FIGURE 5A is an illustration identifying the phospho-Thr 325 peptide used to generate the phospho-Thr-325-specific anti-c-Fos antiserum. FIGURE 5B is a Western blot of NIH 3T3 cells that were transfected with the indicated c-Fos alleles or with vector alone. Extracts were prepared from quiescent (-) or EGF-stimulated (+) cells and analyzed using either the anti-c-Fos antibody or the phospho-Thr 325 antiserum. Results shown are representative of three

independent experiments. FIGURE 5C is a Western blot of  $\Delta$ B-Raf-ER NIH 3T3 cells transfected with Fos-WT or Fos-AA that were either starved and left untreated (0) or treated with 1  $\mu$ M tamoxifen (TAMX) for the indicated times before lysis. The *in vivo* phosphorylation of Thr 325 in Fos-WT and Fos-AA was  
5 analyzed by western blotting using the phospho-Thr 325-specific antiserum. FIGURES 5D and 5E are Western blots demonstrating the *in vivo* mitogen-regulated phosphorylation of Thr 325 in the context of the Fos DEF domain mutants. NIH 3T3 cells expressing the indicated Fos proteins were treated as in the same manner as for FIGURE 3B, and extracts analyzed for phosphorylation of  
10 Thr 325.

FIGURE 6A is a Western blot of quiescent Swiss 3T3 cells were treated with EGF (25 ng/ml) for the indicated times. Lysates were probed for endogenous c-Fos, Thr 325 phosphorylation in c-Fos. FIGURE 6B is a Western blot of Swiss 3T3 cells that were treated with PDGF-BB (20 ng/ml) and processed as in  
15 described for Figure 6A. Results shown are representative of three experiments. FIGURE 6C is a Western blot of quiescent Swiss 3T3 cells that were treated with PDGF for 60 min and then treated with UO126 (5  $\mu$ M), as indicated, or with DMSO (lanes 3-8) for the remainder of the experiment. The expression and phosphorylation of endogenous c-Fos was visualized as in above. FIGURE 6D is  
20 an autoradiogram showing the kinase activities of endogenous ERK1/2 and RSK in cell lysates from Figure 6C. The fold activity is provided above each lane.

FIGURE 7A is a bar graph showing the AP-1 transcriptional activity of the indicated c-Fos alleles in Hela cells. AP-1 luciferase activity in cells expressing Fos-WT is expressed as 100%, and the data shown are from five individual  
25 experiments. FIGURE 7B is a photomicrograph showing the expression of endogenous c-Fos in quiescent (-) or serum-stimulated (+) pMV7-infected 208F fibroblasts (vector), assayed by immunofluorescence microscopy. To examine the

expression of Fos-WT, Fos<sup>Y45A</sup> or Fos<sup>T325A/T331A</sup> in G418-resistant 208F fibroblasts, cells were serum-starved for 24 h before fixation and processing for immunofluorescence microscopy using the anti-Fos antibody. FIGURE 7C is a Western blot of c-Fos protein expression in quiescent 208F cells. FIGURE 7D is a bar graph showing the anchorage-independent growth of G418-resistant pools of 208F cells stably expressing pMV7 (vector) or the indicated FLAG-Fos alleles. The data are expressed as a percentage of the number of colonies formed by cells expressing Fos-WT (100%) and represent the mean  $\pm$ SEM from six experiments performed in duplicate.

FIGURE 8 is a schematic diagram of the molecular interpretation of ERK1/2 signal duration. Growth factor stimulation (stimulus) causes activation of signaling pathways (signals) that result in rapid transcriptional induction of immediate early genes (response). The duration of ERK1/2 signaling is then interpreted by immediate early gene products that contain DEF domains (signal sensors). ERK1/2-docking to the DEF domain results in sensor phosphorylation. Docking and phosphorylation alters its biological activity, and this dictates the biological outcome. TF, transcription factor.

FIGURE 9A is a photomicrograph showing the nuclear accumulation of active ERK1/2 and c-Fos in growth factor-treated Swiss 3T3 cells. These photomicrographs are enlargements of the images of Figure 1C in order to visualize the cellular distribution of activated ERK1/2 and nucleolar structures. FIGURE 9B is a photomicrograph of quiescent Swiss 3T3 cells treated with PDGF for 75 minutes followed by the addition of cyclohexamide (+) or vehicle (-). Cells were processed for c-Fos immunofluorescence 90, 180 or 300 minutes after PDGF stimulation. In control experiments (bottom two panels), cells were incubated with cyclohexamide or vehicle for 20 minutes prior to treatment with PDGF for 90 minutes.

FIGURE 10A is a Western blot of NIH3T3 cells transiently transfected with FosWT or FosDD were left quiescent or treated with EGF (50 ng/ml, 5 min) prior to lysis. An aliquot from each cell extract was incubated in the presence or absence of  $\lambda$  protein phosphatase (P'ase) for 30 minutes on ice. Data shown is  
5 representative of three separate experiments. FIGURE 10B is a Western blot of NIH3T3 cells stably expressing  $\Delta$ B-Raf:ER that were transfected with FosWT, FosAA or FosDD. Cells were deprived of serum growth factors, pre-treated with 5  $\mu$ M UO126 (+) or 0.1% DMSO (-) for 30 minutes prior to treating with tamoxifen (TAMX, 1  $\mu$ M) for 15 minutes prior to cell lysis. FIGURE 10C is a Western blot  
10 showing the phosphorylation of (His)<sub>6</sub>-FosWT, AA or EE or MBP by Flag-ERK5. Active (+) and inactive ERK5 (-) was obtained by coexpressing Flag-ERK5 and HA-MEK5(D) or control vector, respectively, in 293 cells followed by immunoprecipitation of Flag-ERK5 from cell lysates using the M2 anti-Flag monoclonal antibody. Autophosphorylation (auto-P) of ERK5 in kinase reactions  
15 is indicated. Together, these data demonstrate that the phosphorylation of primed c-Fos is regulated by the Raf/Mek/ERK pathway.

FIGURE 11 is a Western blot of quiescent Rat-1 cells that were treated with the indicated concentrations of LPA for various times. The activation kinetics of ERK1/2 demonstrates that c-Fos is a sensor for sustained ERK1/2 signaling in Rat-  
20 1 fibroblasts. The data shown is representative of at least three individual experiments.

FIGURE 12 is a series of cell culture plates, fixed and then stained with Giemsa to visualize foci. The indicated Fos proteins were stably expressed in 208F cells and cultured for four weeks in regular culture medium. Identical data  
25 was obtained from five separate experiments. Thus, substituting aspartic acid for T235 and T331 in c-Fos promotes Fos-mediated transformation.

FIGURES 13A-C are a series of Western blots showing the regulation of ectopically expressed Fos family proteins (c-Fos, Fra-1, and Fra-2) by the ERK1/2 pathway in NIH 3T3 cells. Cells were treated with or without EGF (50 ng/mL) for 5 minutes prior to cell lysis. Where indicated, UO126 (5 mM) was added to cells 30 minutes before adding EGF. EGF treatment in the absence of UO126 activated ERK1/2, as demonstrated by the mobility shift to a higher molecular weight. ERK1/2 activation resulted in phosphorylation of the target amino acid, T325, of c-Fos (Figure 13A). ERK1/2 activation of Fra-1 (Figure 13B) and Fra-2 (Figure 13C) is also demonstrated by the observed mobility shift.

FIGURE 14A is a sequence alignment of c-Fos, Fra-1, and Fra-2 (SEQ ID NO: 25-27, respectively) demonstrating a high degree of sequence identity in the C-termini. Fra-1 and Fra-2 have ERK1/2 and RSK priming phosphorylation sites in addition to DEF domains. FIGURE 14B is a Western blot of NIH 3T3 cells transfected with the indicated constructs and deprived of serum growth factors for 24 hours. These results demonstrate that mutations in the DEF domains of Fra-1 and Fra-2 inhibit the ERK1/2-mediated mobility shift (compare to Figures 13B and 13C).

FIGURE 15A is a Western blot of c-Myc immunoprecipitation from NIH 3T3 cells transfected with pcDNA3 (vector) or c-Myc and deprived of serum growth factors. EGF and UO126 were used to treat cells as described in Figure 13. FIGURE 15B is a Western blot from cells transfected with the indicated c-Myc proteins. These results characterize the DEF domain in c-Myc and show that S62 phosphorylation depends on an intact DEF domain.

FIGURES 16A-F are Western blots demonstrating the kinetics of immediate early gene expression in Swiss 3T3 cells. Cells were deprived of serum growth factors and treated with EGF (25 ng/mL) or PDGF-BB (20 ng/mL) for the indicated times.

FIGURES 17A-B are Western blots demonstrating the kinetics of Egr-1, JunB, and c-Myc expression in Swiss 3T3 cells. Cells were treated as described in Figure 13. Total levels of c-Myc (Figure 17B) were detected by immunoprecipitating c-Myc prior to Western analysis.

5        FIGURES 18A-E are Western blots demonstrating that sustained expression of immediate early genes requires ERK1/2 activity. Serum deprived Swiss 3T3 cells were treated with PDGF-BB for 90 minutes before adding DMSO vehicle (0.1%) or UO126 (5  $\mu$ M).

10        FIGURE 19A is a Western blot of cells treated with PDGF-BB for either 90 minutes (lanes 2-9) or 120 minutes (lanes 11-13) before adding DMSO vehicle (lanes 3-5) or UO126 (lanes 7-9, 12, 13). FIGURE 19B is a Western blot of cells treated with PDGF-BB for 5 hours to induce Fra-1 before adding UO126 for a further 20 or 30 minutes. These figures demonstrate that ERK1/2 signaling is required during G1 for the stabilization of c-Myc.

15        FIGURE 20 is a series of Western blots from Swiss 3T3 cells treated with various concentrations of PDGF-BB before lysis. These results demonstrate that IEG products act as sensors for subtle differences in ERK1/2 signal duration.

20        FIGURE 21A is a bar graph of the result from an *in vitro* kinase assay demonstrating ERK 1/2 activation is sensitive to small differences in growth factor (PDGF) stimulation. FIGURE 21B is a Western blot demonstrating that the c-Fos stabilization observed in Figure 20 following stimulation with 10 ng/ml PDGF is a result of ERK 1/2-dependent phosphorylation of T325. Neither long-term c-Fos stabilization (see Figure 20) nor T325 phosphorylation is observed following 4 ng/ml PDGF stimulation.

25        FIGURE 22 is a series of Western blots showing Fra-1 hyperphosphorylation throughout G1 requires ERK1/2 signaling.

FIGURE 23 is representative gel and the densitometric quantification of an electrophoretic mobility shift assay (EMSA) for AP-1. Swiss 3T3 cells were treated as indicated and extracted in a hypotonic lysis buffer. The nuclear fraction was isolated and aliquots mixed with a  $^{32}\text{P}$ -labelled AP-1 oligonucleotide in a standard EMSA. These results demonstrate that PDGF, but not EGF, treatment significantly increases AP-1 expression and AP-1 DNA binding.

FIGURE 24 is an immunoprecipitation of extracts from 208F cells stably expressing c-Myc or c-Myc F196A and treated with cycloheximide (14 mg/mL) for the indicated times. Following immunoprecipitation of the c-Myc proteins, total levels of c-Myc were detected using Western analysis. These results demonstrate that c-Myc stability requires the DEF domain.

FIGURE 25 is a series of indirect immunofluorescence photomicrographs demonstrating typical results of the screening assays described herein. Representative fields of view using a 10X objective lens are shown.

FIGURE 26 is a series of Western blots and accompanying densitometric analysis showing the effect of mutating the DEF domain binding site in ERK1/2 on a RSK (non-DEF domain-dependent) phosphorylation and c-Fos (DEF domain-dependent) phosphorylation. The bar graphs represent the raw densitometric analysis, unadjusted for ERK1/2 content.

### Detailed Description

We have discovered that DEF domains are present in numerous proteins that are important in a variety of human diseases and, by blocking the interaction of a MAP kinase with the DEF domain of a target protein, effective therapy may be provided. Also provided are screening methods for identifying novel therapeutics that inhibit the MAP kinase-DEF domain interaction. This invention provides several advantages over known therapies that directly target the MAP

kinase signaling cascade. Typically, most compounds that inhibit the MAP kinase pathway are non-specific and inhibit more than one enzyme. Also, the targeted kinases, if effectively inhibited, are not available to perform normal physiological functions necessary for cell survival, resulting in toxicity to healthy as well as  
5 diseased cells. By contrast, the therapeutic methods of the present invention inhibit the activation of particular target proteins, leaving the MAP kinases enzymatically active and available to phosphorylate other, non-DEF domain-containing proteins. Diseased cells (e.g., cancerous cells) are often more  
10 susceptible to therapy because of the higher concentration of target protein, improving the likelihood of success for this approach.

The principles of the invention are exemplified using the immediate early gene, c-Fos, but is not intended to be limiting. c-Fos functions as a molecular sensor for the duration of extracellular-signal-regulated kinase 1/2 (ERK1/2) signaling. c-Fos is known to be phosphorylated by ERK1/2 and RSK, resulting in  
15 increased stability of the protein. Therefore, the biological function of c-Fos differs under conditions where ERK1/2 signaling is sustained, rather than transient. Signaling is transduced by ERK1/2 binding to the DEF domain of c-Fos. Mutating the DEF domain inhibits c-Fos-mediated signaling and, ultimately, the downstream effects of ERK1/2 activation.

20 Further, Fos, Myc and Jun family proteins are transcription factors encoded by immediate early protooncogenes. Family members c-Fos, Fra-1, Fra-2, c-Myc, N-Myc, JunD, and JunB are frequently found to be amplified or upregulated in human cancers. Sustained ERK1/2 signaling is required for cell proliferation and ERK1/2 docking to these proteins occurs only when signaling is sustained.  
25 Docking controls the growth-promoting function of these transcription factors.

Accordingly, ERK1/2 docking inhibitors may be clinically useful drugs because they will likely to inhibit a specific branch of ERK1/2 signaling and would, therefore, be less toxic than general ERK1/2 inhibitors.

## 5 Sustained ERK1/2 Activation Correlates With S Phase Entry

Treatment of quiescent Swiss 3T3 fibroblasts with platelet-derived growth factor (PDGF) stimulated S phase entry; whereas, treatment with epidermal growth factor (EGF) did not (Figure 1A). The activation kinetics and amplitude of ERK1/2 and RSK, however, were almost identical following a 5-10 minute  
10 exposure to either PDGF or EGF (Figure 1B). In both cases, hyperphosphorylated (active) ERK1/2 was localized to the nucleus (Figure 1C). In contrast to ERK1/2 and RSK activation kinetics, rates of inactivation were faster in cells treated with EGF compared to PDGF (Figure 1B). ERK1/2 signaling remained elevated for at least 240 minutes following PDGF exposure, but returned to basal levels within  
15 30-45 minutes following EGF withdrawal (Figure 1B). Notably, the sustained ERK1/2 activity elicited by PDGF treatment remained localized to the nucleus (Figures 1C and 9A), demonstrating a tight correlation with S phase entry. Further, c-Fos protein expression was prolonged in cells treated with PDGF compared to those treated with EGF (Figure 1D). This indicates that c-Fos  
20 becomes stabilized when ERK1/2 signaling is prolonged, but is unstable when ERK1/2 signaling is transient. c-Fos expression was not affected by either the addition of cycloheximide to cells 75 minutes after PDGF treatment (Figure 9B) or the addition of actinomycin D 20 minutes after PDGF treatment. Thus, the differences in c-Fos expression between PDGF- and EGF-treated cells arises from  
25 post-translational control. This conclusion is further supported by studies showing that the transcriptional induction of *c-fos* and other IEGs by various growth factors is completed within 30-45 minutes.

ERK1/2 and RSK coordinately phosphorylate the extreme C-terminus of c-Fos at Ser 374 and Ser 362, respectively (Figure 2A). Mutating these residues to aspartate (Fos-DD), which mimics phosphorylation, results in enhanced transformation of fibroblasts by comparison to c-Fos having Ser 362 and Ser 374 mutated to alanine (Fos-AA) (Okazaki *et al.*, *EMBO J.*, 14: 5048-5059, 1995; Chen *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 10952-10956, 1993). Thus, increasing the stability of c-Fos is not the only manner in which this transcription factor can regulate cellular transformation. Fos-AA appears to be differentially regulated compared to Fos-DD.

We have discovered that the addition of serum to fibroblasts results in a large,  $\lambda$  phosphatase-sensitive electrophoretic mobility shift of Fos-DD, compared to Fos-AA (Figures 2B left, and 10A). This effect correlates with increased incorporation of  $^{32}\text{P}$ -orthophosphate that was consistently two to threefold greater for Fos-DD than Fos-AA (Figure 2B, right). This demonstrates that phosphorylation of Ser 362 and Ser 374 prime c-Fos for additional growth factor-regulated phosphorylation. As Fos-DD has greater transforming potential than Fos-AA, the regulation of primed c-Fos is critical for promoting fibroblast proliferation.

## Phosphorylation of Primed c-Fos is MEK-dependent

NIH 3T3 cells transfected with different Fos proteins were treated with the MEK inhibitor UO126 (Favata *et al.*, *J. Biol. Chem.*, 273: 18623-18632, 1998) to determine if the mitogen-regulated phosphorylation of Fos-DD is mediated by the Raf/MEK/MAPK pathway. UO126 inhibited the growth factor-regulated mobility shift of Fos-WT and Fos-DD (Figure 2C) and ERK1/2 activation (Figure 2C, bottom), indicated that ERK1/2 or downstream signaling molecules regulated primed c-Fos. Identical observations were made using NIH 3T3 cells expressing a

conditionally active form of B-Raf ( $\Delta$ B-Raf-ER) and treating these cells with tamoxifen instead of EGF (Figure 10B). To determine whether ERK1/2 could phosphorylate primed c-Fos *in vitro*, we used different hexahistidine-Fos fusion proteins as substrates. ERK1/2 efficiently phosphorylated Fos-WT (Figure 2D).  
5 The phosphorylation of Fos-AA and primed c-Fos, Fos-EE (S362E/S374E), by ERK1/2 was also easily detected *in vitro*, but the phosphorylation of Fos-EE compared with Fos-AA was consistently greater (Figure 2d). These results demonstrate that ERK1/2 can phosphorylate sites in c-Fos other than Ser 374 and that this phosphorylation is enhanced after priming of the C terminus. In contrast  
10 to ERK1/2, phosphorylation of c-Fos by ERK5 *in vitro*, another UO126-sensitive proline-directed kinase, was not observed (Figure 10C).

#### **An ERK1/2 Targeting Motif Promotes the Phosphorylation of Primed Fos**

The preference of ERK1/2 for primed c-Fos (Fos-EE) over Fos-AA  
15 demonstrates that C-terminal phosphorylation exposes additional phosphorylation sites and/or a motif that would increase the efficiency of phosphorylation at these sites. Examination of the c-Fos sequence identified a site in the C terminus that has similarity with the ERK1/2 targeting motif, FXFP (SEQ ID NO: 1), known as a DEF domain. In c-Fos, this motif is FTYP (Figure 3A; SEQ ID NO: 2).  
20 Mutating either Phe 343 or Tyr 345 to alanine dramatically inhibited the phosphorylation of primed c-Fos (Fos-EE) by ERK1/2 *in vitro* (Figure 3A). ERK1/2-regulated phosphorylation of substrates that contain DEF domains can be competitively inhibited *in vitro* with a synthetic peptide encompassing the DEF domain found in ELK-1. This peptide (FQFP; SEQ ID NO: 3) inhibited the  
25 phosphorylation of primed c-Fos in a concentration-dependent manner (Figure 3B). By contrast, a peptide with a mutant DEF domain (AQAP; SEQ ID NO: 4) was less efficient in inhibiting ERK1/2-mediated phosphorylation of primed c-Fos.

The ELK-1 peptide was then engineered to contain the c-Fos FTYP DEF domain (SEQ ID NO: 2). This peptide also inhibited primed c-Fos phosphorylation, but a mutant version (ATYP; SEQ ID NO: 5) did not (Figure 3C). In both cases, the IC<sub>50</sub> for the FQFP (SEQ ID NO: 3) and FTYP (SEQ ID NO: 2) peptides was approximately 80  $\mu$ M. The EGF-stimulated mobility shift of Fos-DD *in vivo* was also inhibited when Phe 343 or Tyr 345 were mutated to alanine (Figure 3D). These results demonstrate that the initial phosphorylation of c-Fos by ERK1/2 and RSK as the extreme C terminus expose an ERK1/2 docking site that allows ERK1/2 to phosphorylate additional sites.

Based on this model, mutation of Phe 343 or Tyr 345 to alanine should prevent hyperphosphorylation of Fos-WT and not interfere with the priming phosphorylations, which are C-terminal to the DEF domain. Indeed, these mutations prevented the appearance of the slowest mobility, but still allowed a shift to the intermediate mobility (Figure 3E). This indicates that the DEF domain is not involved in directing ERK1/2 to prime c-Fos through Ser 374 phosphorylation. Instead, ERK1/2 docking through the DEF domain results in the hyperphosphorylation of primed c-Fos.

#### **ERK1/2 phosphorylates Thr 325 and Thr 331 in primed c-Fos**

There are two proline-directed threonine residues (Thr 325 and Thr 331) amino-terminal to the DEF domain in c-Fos (Figure 4A). Mutation of Thr 325 to alanine almost completely inhibited the phosphorylation of Fos-EE by ERK1/2 *in vitro*, and the additional mutation of Thr 331 to alanine was required to reduce the phosphorylation to background levels (Figure 4A). Thr 325 and Thr 331 were also phosphorylated in primed c-Fos (Fos-DD) *in vivo*, as evidenced by the complete loss of the mobility shift in the T325A/T331A mutant (Figure 4B). Individual mutation of Thr 325 or Thr 331 to alanine in the context of Fos-DD only partially

inhibited growth factor-regulated phosphorylation. Substituting alanines for Thr 325 and Thr 331 in the context of Fos-WT prevented the EGF-stimulated to the slowest mobility (Figure 4C). However, EGF treatment was associated with the appearance of the intermediate mobility form, resulting from priming  
5 phosphorylation of ERK1/2 and RSK. Collectively, these observation demonstrate an ordered phosphorylation process whereby the initial phosphorylation of c-Fos at Ser 374 and Ser 362 (priming) exposes a DEF domain that mediates the hyperphosphorylation of c-Fos at Thr 325 and Thr 331. Further, Ser 374 phosphorylation is not regulated by docking. This is consistent with the  
10 phosphorylation of amino acids N-terminal, but not C-terminal, to DEF domains.

A phosphorylation-specific antiserum for Thr 325 in c-Fos (Figure 5A) was generated to investigate the mitogen-regulated phosphorylation of this residue in primed c-Fos. The antiserum showed little or no reactivity with Fos-WT or Fos-DD expressed in quiescent cells (Figure 5B, -EGF). However, after treatment with  
15 EGF, strong reactivity was associated with Fos-WT and Fos-DD, but not with Fos<sup>T325A</sup> or Fos-DD<sup>T325A</sup> (Figure 5B, +EGF). Priming of the extreme C terminus by ERK1/2 and RSK promotes additional phosphorylation of c-Fos *in vivo* (Figure 2B). To determine if this is caused by increased phosphorylation of Thr 325, Fos-WT and Fos-AA were expressed to similar levels in the ΔB-Raf-ER NIH 3T3 cells  
20 that were then treated with tamoxifen for varying times (Figure 5C). The phosphorylation of Thr 325 was greater in cells transfected with Fos-WT than those transfected with Fos-AA.

Mutating Phe 343 or Tyr 345 to alanine prevented the hyperphosphorylation of primed c-Fos (Figure 3). Specifically, the regulation of Thr 325  
25 phosphorylation *in vivo* was inhibited when Phe 343 or Tyr 345 were mutated to alanine, either in the context of Fos-WT (Figure 5D) or Fos-DD (Figure 5E).

In this later experiment, there is a strong correlation between the mobility shift of Fos-DD and increased Thr 325 phosphorylation confirming that the DEF domain in c-Fos increases the efficiency of Thr 325 phosphorylation *in vivo*.

**5 The phosphorylation of Thr 325 is differentially regulated by ERK1/2-signal duration**

As shown above, the induction kinetics of c-Fos expression 30-45 min after addition of PDGF or EGF to Swiss 3T3 cells were identical (Figure 1D). This is consistent with a model in which an initial activation of ERK1/2 or RSK is  
10 sufficient for induction of *c-fos* IEG expression. To determine if ERK1/2 signal duration differentially regulates the phosphorylation of Thr 325 in endogenous c-Fos, we prepared extracts from Swiss 3T3 cells treated with EGF or PDGF for different times. Importantly, although c-Fos is present in cells after 45 or 60 min of EGF treatment, Thr 325 phosphorylation was not observed (Figure 6A). This is  
15 consistent with inactivation of ERK1/2 occurring before c-Fos is present (Figure 6A). By contrast, phosphorylation of Thr 325 increased 45-60 min after addition of PDGF (Figure 6B). Maximal Thr 325 phosphorylation persisted for at least 120 min (Figure 6B), but was still detected after 240 min. In Rat-1 fibroblasts, treatment with 100  $\mu$ M lysophosphatidic acid (LPA) results in sustained ERK1/2  
20 activity and S phase entry, whereas treatment with 0.1-1  $\mu$ M LPA transiently activates ERK1/2 and no cell cycle progression occurs. Although treatment of quiescent Rat-1 fibroblasts with mitogenic (100  $\mu$ M) and no-mitogenic (0.5  $\mu$ M) concentrations of LPA resulted in a similar induction of *c-fos*, phosphorylation of Thr 325 only occurred with 100  $\mu$ M LPA (Figure 11). These findings correlate  
25 with the generation of transient and sustained ERK1/2 responses by 0.5  $\mu$ M and 100  $\mu$ M LPA, respectively (Figure 11).

Thus, differential phosphorylation of c-Fos occurs in different cell types and in response to agonists that directly activate tyrosine kinase receptors or heterotrimeric G protein-coupled receptors.

To show that the sustained phase of ERK1/2 signaling was required to  
5 mediate the stabilization and hyperphosphorylation of endogenous c-Fos in Swiss 3T3 cells, ERK1/2 and RSK activity was inhibited by adding UO126 to cells that had been treated with PDGF for 60 min (Figure 6D). Under this condition, the phosphorylation of Thr 325 was completely inhibited and the electrophoretic mobility of c-Fos increased (Figure 6C). Importantly, these UO126-induced  
10 changes in the biochemical properties of c-Fos also preceded the rapid disappearance of c-Fos protein. This result is consistent with hypophosphorylated c-Fos being unstable. These observation show that the phosphorylation of c-Fos at Thr 325 is tightly correlated with the activation/inactivation kinetics of ERK1/2 in different cell types of provide clear evidence that c-Fos can function as sensor for  
15 ERK1/2 signal duration.

#### **The DEF domain and Thr 325/Thr 331 phosphorylation modulates c-Fos function**

In Hela cells expressing Fos-WT, AP-1 transcription factor activity was  
20 consistently three to fourfold above background levels (Figure 7A). Mutation of Thr 325 and Thr 331 to alanine reduced AP-1 activity by about 20%; whereas, mutating the DEF domain (F343A) reduced Fos-WT activity by about 65% (Figure 7A). These observations indicate that docking of ERK1/2 to c-Fos is important in regulating c-Fos transcriptional activity under conditions of growth factor  
25 stimulation. To determine if ERK1/2 docking to c-Fos can contribute to c-Fos function independently of the phosphorylation-mediated stabilization, Fos-WT, Fos<sup>T325A/T331A</sup> or Fos<sup>Y345A</sup> (a DEF domain mutant) were stably expressed in 208F

fibroblasts. The expression of the different Fos proteins (Figure 7B, bottom six panels) was equivalent to the level of endogenous c-Fos in serum-stimulated vector-infected cells (Figure 7B, top two panels) and was also localized to the nucleus. Western analysis of c-Fos expression in the quiescent cell lines also  
5 showed that they were expressed to similar levels (Figure 7C). The stable expression of Fos-WT promoted anchorage-independent growth in soft agar (Figure 7D), as expected. Mutating Thr 325 and Thr 331 to alanine significantly reduced the growth of 208F cells in soft agar suggesting that phosphorylation of these residues promotes cellular transformation (Figure 7D). However, replacing  
10 Thr 325 and Thr 331 with Asp enhanced c-Fos-mediated focus formation (Figure 12). Further, mutating the c-Fos DEF domain (Fos<sup>Y345A</sup>) completely inhibited the ability of c-Fos to transform 208F cells; more so than the c-Fos<sup>T325A/T331A</sup> mutant (Figure 7D). These results demonstrate that ERK1/2 docking to c-Fos contributes to transformation through mechanisms in addition to Thr 325/Thr 331  
15 phosphorylation and that stabilization of c-Fos is not the only factor that regulates c-Fos function, as all proteins were expressed equally. In addition, it also demonstrates that ERK1/2 docking to c-Fos regulates biological activity.

#### **Mutating the DEF domain binding site inhibits target residue 20 phosphorylation**

In order to confirm the criticality of ERK1/2 docking with the DEF domain to phosphorylation of a target residue, the DEF domain binding site in ERK1/2 was mutated. Six single mutations in ERK1/2 (L198A, Y231A, L232A, L235A, Y261A, and D319N) were expressed in NIH 3T3. Several of these mutations have  
25 been previously described as forming the DEF domain binding site in ERK1/2 (Lee *et al.*, *Molec. Cell*, 14: 43-55, 2004). Each ERK1/2 mutant was tagged with HA for later detection. EGF-stimulated kinase activity of wildtype and mutant

ERK1/2 was measured using RSK, a non-DEF domain-containing target protein, and c-Fos, DEF domain-containing target protein, as substrates in a standard  $^{32}\text{P}$ -phosphorylation assay. Fos phosphorylation by ERK1/2 mutants with disrupted DEF domain binding pockets (L198A, Y231A, L232A, L235A, and Y261A) was almost completely absent (Figure 26). By contrast, RSK phosphorylation—a event that does not require ERK1/2 interaction with a DEF domain—was only moderately reduced (Figure 26).

These results demonstrate that DEF domain binding is required for a target residue phosphorylation but that a disruption of the DEF domain inhibition does not abolish all kinase activity. Thus, the DEF domain binding event is separate and distinct from the phosphorylation event. Disruption of DEF domain binding may be used to selectively inhibit phosphorylation of a target protein, without significantly inhibiting the phosphorylation of non-target proteins (i.e., proteins that do not contain a DEF domain) by the same kinase. Thus, small molecule inhibitors and polypeptide inhibitors (e.g., naked DEF domains) which specifically inhibit DEF domain binding are useful for selectively inhibiting the phosphorylation of target proteins without causing the adverse effects associated with complete inhibition of a target kinase (e.g., a MAP kinase).

## 20 Mechanism of IEG Activation Through DEF Domain Binding

The mechanism described here employs an IEG product, typified by c-Fos, which functions as a molecular sensor that differentiates between differences in ERK1/2 and RSK signal duration, as well as their cytoplasmic/nuclear distribution (Figure 8). As observed for a large number of IEGs, the *c-fos* gene is transcriptionally induced within minutes of growth factor stimulation and therefore occurs with kinetics that are independent of differences in signal duration. Newly synthesized c-Fos protein has a half-life of about 30-45 min but, when

phosphorylated by ERK1/2 and RSK, the half-life is extended to at least 2h. Thus, when ERK1/2 is rapidly activated (transient signal), c-Fos is present in the nucleus, but is not phosphorylated, and is therefore unstable and degraded (Figure 8). By contrast, delayed inactivation of ERK1/2 (sustained signal) results in the efficient phosphorylation of c-Fos at its extreme C terminus, resulting in its stabilization for several hours. The initial priming phosphorylation in the C-terminus exposes a DEF domain that promotes additional ERK1/2-mediated phosphorylation events, increasing the efficiency of ERK1/2-regulated phosphorylation when ERK1/2 is only sub-maximally active (0.5-4 h after stimulation). Further, when priming and docking are inhibited by point mutation (Fos-AA and Fos<sup>F343A</sup> or Fos<sup>Y345A</sup>, respectively) ERK1/2 and/or RSK signals are unable to alter c-Fos function. These non-phosphorylatable c-Fos mutants likely resemble the hypophosphorylated form of c-Fos that is present when ERK1/2 is rapidly inactivated during transient signaling and cells do not enter S phase.

Simply prolonging the half-life of c-Fos will not affect its role in promoting transformation. Instead, the combination of protein stabilization and DEF-mediated regulation allows c-Fos to function as sensor for ERK1/2. If c-Fos is not stabilized during the sustained phase of signaling, ERK1/2 will not target the c-Fos DEF domain. Therefore, stabilizing the IEG product is a critical first step if it is to function as a sensor for sustained ERK1/2 signals. The physiological importance of the c-Fos DEF domain is underscored by the fact that mutations in the DEF domain significantly reduced AP-1 activity and inhibit the transforming activity associated with wild-type c-Fos. However, the effect of mutating the DEF domain is stronger than the effect of mutating the phosphorylation sites that are controlled by this docking site, indicating that the DEF domain can have more than one action. An additional function of the DEF domain ERK1/2-mediated *trans*-phosphorylation of AP-1 complex proteins.

We described a general mechanism for cellular sensing of ERK1/2 signal strength and timing involving the FTYP (SEQ ID NO: 2) DEF domain present in many IEGs. Putative DEF domains are found in additional AP-1 proteins, such as Fra-1, Fra-2, Jun-B and JunD (Table 1). The proto-oncogene products c-Myc and N-Myc also contain putative DEF domains. The IEG product Egr-1 has a DEF domain and several putative proline-directed phosphorylation sites N-terminal to this domain that could enable Egr-1 to sense sustained signaling in PC12 cells and promote neuronal differentiation. In common with the c-Fos DEF domain, the other DEF domains highlighted in Table 1 show subtle deviation from the FXFP consensus (SEQ ID NO: 1), with respect to the presence of phenylalanine at positions 1 and 3 indicating that tyrosine can be tolerated at either site.

Table 1. DEF Domains in Immediate Early Gene Products		
<i>IEG</i>	<i>Amino Acid Sequence</i>	<i>SEQ ID NO.</i>
c-Fos	<sup>314</sup> -GPMVTELEPLCTP-VVTCTPSCTTYTSSFVFTYPEEADS	6
Fra-1	<sup>211</sup> -GP-VLEPEALHTPTLMT-TPSLTPFTPSLVFTYPSTPEP	7
Fra-2	<sup>169</sup> -GGFYGE-EPLHTP-IVVTSTPAITPGTSNLVFTYPSVLEQ	8
Fos-B	<sup>283</sup> -HSEVQV-LGDPFPVV-SPS-YTSSFVLTCPEVSAF	9
JunD	<sup>87</sup> -LLASPDGLLLKLASPELERLIQSNGLVTTTPTST-QFLYPKV	10
JunB	<sup>71</sup> -GQGSDTGASLKLASSELERLIVPNSNGVITTTPTPPGQYFYPRG	11
c-Jun	<sup>60</sup> -LLTSPDVGLLLKLASPELERLIQSSNGHITTTPTPT-QFLCPKN	12
c-Myc	<sup>56</sup> -LPTPPLSPSRRSGLCSPSYV	13
	<sup>181</sup> -LTA-AASECIDPSVVFYPLND	14
N-Myc	<sup>77</sup> -AQSPGAGAASPAGRGHGAAGA	15
	<sup>110</sup> -AHPAAECVDPAVVFPFPVNK	16

Egr-1	<sup>184</sup> -QSPPLSCAVPSNDSSPIYSAAPTFTPNTD	17
	<sup>247</sup> -PMIPDYLFPPQ	18
MPer1	<sup>697</sup> -PRGGPQPLPPAPTSVPPAAFPAPLVTPMVALPNYLFPTPSSY	19
DEF domains are in bold and number indicate amino acid position. Sequences are from rat (c-Fos, Fra-1, and Fra-2), mouse (FosB, JunD, c-Jun, c-Myc, and Egr-1), or human (JunB, N-Myc, and mPer1).		

### Screening Methods to Identify Inhibitors of DEF Domain Binding

#### *DEF Domain Binding Assessment using a Phospho-specific Antibody*

We have developed a cell-based assay which is used to screen small molecule compound libraries (Figure 25). In this assay, rat 208F fibroblasts that stably express c-Fos are cultured in a 384-well plate and deprived of serum growth factors for 24 hours. Cells are then treated with EGF for 15 minutes and fixed with 3.7% formaldehyde. Permeabilized fixed cells are incubated with DAPI to stain the nuclei and an anti-phospho-ERK1/2 mouse monoclonal antibody and an anti-phospho-T325 Fos rabbit polyclonal antibody for 2 hours. Anti-mouse Alexa594-conjugated IgG and an anti-rabbit FITC-conjugated IgG are added to each well and unbound antibody is removed by several washes. The fluorescence intensity of both fluorophores in each well can be detected using an automated epifluorescence microscope or Autoscope (Universal Imaging Systems, Inc.). A clear increase in T325 phosphorylation was observed in cells treated with EGF (indicating that ERK1/2 docking to Fos has taken place). In the same population of cells, the phosphorylation of ERK1/2 also increased, thus indicating its activation by EGF. Under these conditions, only background levels of fluorescence were detected when both phospho-specific antibodies were omitted, and the secondary antibodies show no cross-species reactivity.

Inhibition of ERK1/2 docking to the c-Fos DEF domain in vivo could result from compounds that (a) are generally toxic, (b) prevent the activation of ERK1/2, (c) prevent the growth factor regulated translocation of ERK1/2 into the nucleus where c-Fos is localized or (d) directly antagonize ERK1/2 docking to the DEF domain. The assay we have developed naturally excludes the first three possibilities. First, toxicity will be reflected by nuclear integrity as visualized with DAPI staining. Second, inhibition of ERK1/2 activation/activity will be apparent from the phospho-ERK1/2 fluorescence signal. Third, nuclear translocation of ERK1/2 can be verified by manually examining images from wells that show decreased c-Fos phosphorylation. Therefore, candidate compounds that specifically inhibit ERK1/2 binding to the DEF domain are defined as compounds that decrease the phosphorylation of T325 in c-Fos but which have no effect on ERK1/2 activation or its localization. Although this assay is exemplified using rat fibroblasts, it may be performed using any appropriate cell type including, for example, myoblasts, epithelial cells, and hepatocytes.

#### *Interaction-Trap Assays*

A standard yeast two-hybrid assay may be used to assess the effect of a test compound on the MAP kinase-DEF domain interaction (Mendelsohn and Brent, *Curr. Opin. Biotechnol.* 5:482-486, 1994). Typically, a vector encoding a synthetic or naturally occurring peptide containing a DEF domain, covalently bound a DNA binding domain (e.g., GAL4), is transfected into yeast cells containing a reporter gene operably linked to a binding site for the DNA binding domain. Further, a vector encoding either the native MAP kinase of interest, or a synthetic fragment containing the sequence that interacts with the target DEF domain, covalently bound to a transcriptional activator (e.g., GalAD) is also transfected.

The effectiveness of a test compound is then assessed by growing the yeast in the presence of the compound and measuring the level of reporter gene expression.

#### *GST Pulldown Assays*

5       The interaction of a MAP kinase with a DEF domain may be examined using a GST-fusion protein binding study. A vector encoding a naturally-occurring or synthetic polypeptide containing the DEF domain of interest is fused to GST and expressed in a host cell (e.g., *E. coli* or *Saccharomyces spp.*). The GST fusion protein is then contacted with a MAP kinase in the presence and  
10   absence of a test compound. The MAP kinase may be naturally expressed by the host cell or may be expressed from a second vector inserted into the host cell. Following incubation with the test compound, the host cells are lysed and the GST fusion proteins are recovered using glutathione-Sepharose (GSH-Seph) beads. Typically, the GST fusion proteins are released from the GSH-Seph by boiling and  
15   the proteins visualized by electrophoretic separation on an SDS-PAGE gel. A skilled artisan will readily understand that the GST-Pulldown assay described here can be readily adapted to a cell-free assay by incubating the purified GST fusion protein with a purified recombinant MAP kinase.

#### 20       *Fluorescence Polarization Assay*

A variety of well known cell-free techniques may be used to assess the effects of a test compound on the interaction between a MAP kinase and a DEF domain-containing target protein. Fluorescence polarization assays are particularly useful for this purpose. In this assay, a peptide (about 6-12 amino acids)  
25   containing a DEF domain at its C-terminus and a fluorophore (e.g., fluorescein) conjugated to its N-terminus is incubated in the presence and absence of increasing amounts of recombinant MAP kinase (e.g., GST-ERK1; 0.01-1  $\mu$ M) for 10

minutes at room temperature. Aliquots from each reaction are placed in a plate black-walled microtiter (e.g., 384-well) plate and polarization measured using an Analyst plate reader. Increasing concentrations of the MAP kinase causes an increase in polarization. Titrating in the "free" DEF domain-containing peptide  
5 (i.e., un-conjugated) inhibits the change in polarization, whereas the mutated DEF domain peptide does not. The appearance of low polarization, even in the presence of high concentrations of kinase, indicates flexible binding of the DEF domain to the kinase and suggests the presence of the propeller effect. Designing shorter dye-conjugated DEF domain-containing peptides usually alleviates this  
10 problem. The effect of standard assay variables, including incubation time, temperature, pH (7.2-8.5), and buffers, on polarization is readily controlled during routine assay optimization.

This assay is readily adaptable for identifying test compounds that inhibit binding of a MAP kinase to a DEF domain. The use of automated liquid handling  
15 systems and plate readers makes this assay readily adaptable to a high-throughput format for screening large numbers of test compounds. For compound screening, the test compound is added to a mixture of the fluorescently labeled DEF domain-containing peptide and the target MAP kinase. Compounds that inhibit the polarization increase (or cause a decrease in polarization) resulting from increasing  
20 amounts of the MAP kinase are therapeutic candidates.

#### *Identification of Test Compounds as Potential Therapeutics*

We have identified a variety of DEF domain-containing target proteins that have been implicated in a variety of diseases. The particular DEF domain or target  
25 protein may be substituted for c-Fos in any of the exemplary assays described here. Further, the lists of target proteins provided are not intended to be limiting. Other target proteins are easily identified based on the availability of a DEF domain.

Test compounds having antineoplastic activity are those that inhibit binding of a MAP kinase (e.g., ERK 1/2) to the DEF domain of any of the proteins of Table 1 (except mPer1) or Table 2. Test compounds that are useful for treating cardiovascular disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 3. Test compounds that are useful for treating acute and chronic inflammation or inflammatory disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 4. Test compounds that are useful for treating a variety of metabolic disorders inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 5. Test compounds that are useful for treating a variety of nervous system disorders (e.g., central and peripheral neuropathies) and behavioral disorders (e.g., psychosis, schizophrenia, autism, Down's Syndrome, Parkinson's Disease, Alzheimer's Disease, epilepsy, Cockayne syndrome, depression, and opiate addiction) inhibit MAP kinase binding to the DEF domain of the proteins identified in Table 6. Test compounds useful for treating sleep disorders inhibit MAP kinase binding to the DEF domain of the Per proteins (Table 1: mPer; Table 7). Other potentially useful therapeutics inhibit MAP kinase binding to the DEF domain of the PKA-anchoring proteins (AKAPs) (Table 7). In each of Tables 2-7, the alpha-numeric Accession Codes refer to the SWISS-PROT accession numbers. The numeric Accession Codes refer to the GENPEPT accession numbers. In each case, the DEF domain is underscored.

#### **Administration of a DEF Domain Inhibitors or Candidate Compounds for the Treatment of Disease**

As described above, ERK1/2 activate several IEG products through an interaction with the DEF domain and a subsequent phosphorylation event. It is also well known that activation of certain IEGs, and the proteins identified in

Table 2, cause cellular proliferation and may cause tumor promotion and progression. Accordingly, this invention also provides methods and compositions for antineoplastic (i.e., cancer) therapy by administering DEF domain inhibitors. Likewise, therapy for cardiovascular disorders, inflammatory disorders, metabolic disorders, neuropathies and behavioral disorders, and sleep disorders may be provided by inhibiting MAP kinase binding to the DEF domain of one or more of the proteins identified in Table 3, 4, 5, 6, and 7, respectively. Useful DEF domain inhibitors include compounds that bind to the DEF domain of target proteins and prevent the binding of the target kinases. Also, DEF domain inhibitors include “bait” proteins that bind activated target kinases but do not cause cellular proliferation or tumor promotion and/or progression.

In addition to candidate compounds identified using the screening methods of this invention, DEF domain inhibitors can be created by inserting, by artifice, a DEF domain into a non-target protein. The cellular activation/proliferation pathway described herein is limited by the presence of activated target kinase, not by the availability of target proteins. Thus, a DEF domain that is present in a non-target protein effectively “baits” the target kinase, reducing its availability to phosphorylate the target proteins. DEF domains suitable for therapy have the general structure: F/Y—X<sub>1</sub>—F/Y—X<sub>2</sub> (SEQ ID NO: 28). Desirably, X<sub>2</sub> is proline. Most desirably, the DEF domain is identical to the DEF domain of the target protein to which therapy is directed. For example, Figures 3B and C demonstrate that the “naked” DEF domains FQFP (SEQ ID NO: 3) and FTYP (SEQ ID NO: 2) are effective inhibitors of target protein phosphorylation. Substitution of phenylalanine for alanine in these polypeptides results in approximately a two-fold reduction in potency.

Accordingly, therapy can be provided by administering pharmaceutical formulations containing a naked DEF domain. Typically, these polypeptides are administered by parenteral injection such as intravenous, intramuscular, or subcutaneous injection. These small polypeptides may be administered in any appropriate formulation including, for example, in a liposomal formulation. The polypeptides may also be injected directly into a solid tumor.

Alternatively, therapy can be achieved by administering a chimeric protein consisting of a DEF domain that is engineered into a non-target protein. Typically, the chimeric protein will “display” the four amino acid DEF domain on a hydrophilic face, making it available to bind to the activated target kinase. The non-target protein can be chosen based upon the desired pharmacokinetic or pharmacodynamic effect and is readily determined by a person of ordinary skill. For example, a DEF domain inhibitor sequence may be engineered into a serum protein such as albumin or ceruloplasmin in order to prolong the plasma half life. Alternatively, the DEF domain may be engineered into a protein that promotes uptake into a particular cell type.

### ***Pharmaceutical Formulations***

The peptide agents and candidate compounds of the invention can be administered to a subject, e.g., a human, directly or in combination with any pharmaceutically acceptable carrier or salt known in the art. Pharmaceutically acceptable salts may include non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acids such as hydrochloric

acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. One exemplary pharmaceutically acceptable carrier is physiological saline. Other physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences, (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, PA.

Pharmaceutical formulations of a therapeutically effective amount of a peptide agent or candidate compound of the invention, or pharmaceutically acceptable salt thereof, can be administered orally, parenterally (e.g. intramuscular, intraperitoneal, intravenous, or subcutaneous injection), or by intrathecal or intracerebroventricular injection in an admixture with a pharmaceutically acceptable carrier adapted for the route of administration.

Methods well known in the art for making formulations are found, for example, in Remington's Pharmaceutical Sciences (19th edition), ed. A. Gennaro, 1995, Mack Publishing Company, Easton, PA. Compositions intended for oral use may be prepared in solid or liquid forms according to any method known to the art for the manufacture of pharmaceutical compositions. The compositions may optionally contain sweetening, flavoring, coloring, perfuming, and/or preserving agents in order to provide a more palatable preparation. Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier or excipient. These may include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, sucrose, starch, calcium phosphate, sodium phosphate, or kaolin. Binding agents, buffering agents, and/or lubricating agents (e.g., magnesium stearate) may also be used. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and soft gelatin capsules. These forms contain inert diluents commonly used in the art, such as water or an oil medium. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying agents, and suspending agents.

Formulations for parenteral administration (i.e., intravenous, intramuscular, and subcutaneous injection) include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of suitable vehicles include propylene glycol, polyethylene glycol, vegetable oils, gelatin, hydrogenated naphthalenes, and injectable organic esters, such as ethyl oleate. Such formulations may also contain adjuvants, such as preserving, wetting, emulsifying, and dispersing agents. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for the proteins of the invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

Liquid formulations can be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, or by irradiating or heating the compositions. Alternatively, they can also be manufactured in the form of sterile, solid compositions which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

The amount of active ingredient in the compositions of the invention can be varied. One skilled in the art will appreciate that the exact individual dosages may be adjusted somewhat depending upon a variety of factors, including the protein being administered, the time of administration, the route of administration, the nature of the formulation, the rate of excretion, the nature of the subject's conditions, and the age, weight, health, and gender of the patient. Generally,

dosage levels of between 0.1 µg/kg to 100 mg/kg of body weight are administered daily as a single dose or divided into multiple doses. Desirably, the general dosage range is between 250 µg/kg to 5.0 mg/kg of body weight per day. Wide variations in the needed dosage are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected to require higher dosage levels than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, which are well known in the art. In general, the precise therapeutically effective dosage will be determined by the attending physician in consideration of the above identified factors.

The protein or candidate compound of the invention can be administered in a sustained release composition, such as those described in, for example, U.S. Patent No. 5,672,659 and U.S. Patent No. 5,595,760. The use of immediate or sustained release compositions depends on the type of condition being treated and the desired pharmacokinetic profile. For preventive or long-term treatments, a sustained released composition may be preferred.

The protein or candidate compound of the present invention can be prepared in any suitable manner. The protein or candidate compound can be isolated from naturally occurring sources, recombinantly produced, or produced synthetically, or produced by a combination of these methods. The synthesis of short peptides is well known in the art. See e.g. Stewart et al., Solid Phase Peptide Synthesis (Pierce Chemical Co., 2d ed., 1984).

## Methods

### Cell Culture

NIH 3T3 fibroblasts were transfected with Lipofectamine (Invitrogen, Carlsbad, CA) and then cultured for 18 h in DMEM/10% calf serum. Swiss 3T3  
5 fibroblasts expressing a conditionally active form of B-Raf, were cultured in DMEM containing 10% fetal bovine serum (FBS). Before stimulation, cells were cultured in DMEM containing 20 mM HEPES (starving medium) for 24 h (NIH 3T3 or 208F) or 48 h (Swiss 3T3). Rat-1 fibroblasts were cultured for 48 h, washed with starving medium and culture for an additional 24 h in starving  
10 medium. For AP-1 assays, Hela cells were transfected with Lipofectamine for 6 h and then cultured for an additional 16 h before cell lysis and assay of luciferase activity (Promega). EGF and PDGF (Invitrogen) were reconstituted in sterile water containing 0.1% BSA. LPA (Aventi Polar Lipids, Alabaster, AL) was reconstituted in 50% ethanol before sonication for 30 min.

15 Retroviruses used to infect rat 208F cells were produced as described previously (Chen *et al.*, *Oncogene*, 12:1493-1502, 1996). Neomycin-resistant pools of c-Fos-expressing cells were assayed for anchorage-independent growth or focus formation. Metabolic labeling with <sup>35</sup>S-methionine or <sup>32</sup>P-orthophosphate (performed in parallel) was performed as described by Chen *et al.*

20

### Cell lysis and western analysis

Cell extracts were prepared as described previously (Richards, *et al.*, *Curr. Biol.*, 9: 810-820, 1999). To analyze shifts in c-Fos mobility, samples were resolved on a 7.5% SDS-polyacrylamide gel electrophoresis (PAGE) gel,  
25 transferred to nitrocellulose and probed with anti-c-Fos antibody (Upstate Biotechnology Inc., Lake Placid, NY). This antibody is specific for c-Fos and does not cross-react with FosB, Fra-1 or Fra-2. For ERK1/2-MAPK western

analysis, a polyclonal anti-ERK1/2 antibody to an anti-phospho-p42/p44 MAPK monoclonal antibody was used (Sigma, St. Louis, MO). Phosphatase treatment of cell extract was performed for 30 min on ice using  $\lambda$  protein phosphatase (New England Biolabs, Beverly, MA). To generate antiserum specific for  
5 phosphorylated Thr 325 in c-Fos, residues 317-329 (VTELEPLCTPPVVT) (SEQ ID NO: 20) were synthesized (underlined residue is phospho-Thr at position 325), conjugated to keyhole limpet haemocyanin and injected into rabbits (Research Genetics, Inc., Huntsville, AL). To determine the specificity of the antiserum, extracts from cells expressing vector or c-Fos proteins were immobilized on  
10 nitrocellulose and probed with a solution of this anti-serum (1:3000) for 12 h at 4 °C.

#### Recombinant protein purification

M15pREP4 cells transformed with pDS56-(His)<sub>6</sub>Fos or pETHis<sub>6</sub>/ERK2 and  
15 MEK 1 R4F were cultured at 25 °C until an OD<sub>600</sub> of 0.7 was attained. Cells were then incubated in the presence of 1mM isopropyl- $\beta$ -D-thiogalactoside (IPTG) for an additional 12 h at 25 °C. and then harvested by centrifugation. Pellets were resuspended in column buffer (20 mM Tris-HCl at pH 8.0, 200 mM sodium chloride, 10% glycerol, and 10 mM imidazole) and cells were lysed by passage  
20 through a French Press. The (His)<sub>6</sub> proteins were purified using Nickel-NTA-agarose resin (Qiagen, Alencia, CA), dialyzed in column buffer containing 50% glycerol and then stored at -20°C.

### ***In vitro* kinase reactions**

Phosphorylation of (His)<sub>6</sub>-Fos by ERK1 immunoprecipitated from NIH3T3 cells, activate (His)<sub>6</sub>-ERK2 (ref. 42) or FLAG-ERK5/BMK1, was performed in kinase buffer containing 10  $\mu$ Ci  $\gamma$ -<sup>32</sup>P-ATP at 30 °C (Chen *et al.*, *Mol. Cell. Biol.* 10:3204-3215, 1990). Endogenous ERK1 and RSK kinase activities were performed as described previously (Chung *et al.*, *Mol. Cell. Biol.*, 11:1868-1871, 1991). The HPLC-purified synthetic peptides used in the competition kinase assays were mixed with (His)<sub>6</sub>-Fos-EE before addition of activated ERK2 and  $\gamma$ -<sup>32</sup>P-ATP. The peptides derived from ELK-1 were as follows:

10 RRPRSPAKLSFQFPSFQFP (SEQ ID NO: 21); RRPRSPAKLSAQAPSAQAP (SEQ ID NO: 22); RRPRSPAKLSFTYPSFTYP (SEQ ID NO: 23); RRPRSPAKLSATYPSATYP (SEQ ID NO: 24).

### **Immunofluorescence**

15 Swiss 3T3 cells ( $1.35 \times 10^5$  per 35-mm dish) were cultured on poly-L-lysine-coated glass coverslips for 24 h and serum-starved for 48 h. Cyclohexamide (14  $\mu$ g/ml) was delivered in dimethyl sulphoxide. After stimulation with growth factors, cells were washed with ice-cold PBS containing 0.1% BSA, fixed with 3.7% formaldehyde for 10 min at room temperature and permeabilized with 0.2% Triton-X100 for 5 min. Analysis of c-Fos expression was performed using a rabbit anti-human c-Fos antibody (1:500, Upstate Biotechnology Inc.) under conditions described by the manufacturer. Conditions for phospho-p24/p44 MAPK immunofluorescence were identical to those used for c-Fos, except that a monoclonal phospho-MAPK antibody was used (Sigma). Coverslips were mounted in Citifluor (Ted Pella Inc., Redding, CA) and examined under

25 epifluorescent illumination.

**Bromodeoxyuridine (BrdU) incorporation**

Swiss 3T3 cells were cultured as described for immunofluorescence studies, treated with growth factors and 20  $\mu$ M BrdU Labeling Reagent (Amersham Life Sciences Inc., Piscataway, NJ) for 20 h at 37 °C. For immunofluorescence  
5 analysis, a mouse anti-BrdU monoclonal (Amersham Life Sciences Inc.) supplemented with DNAase I (Invitrogen) was used.

**Other Embodiments**

10 All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily  
15 apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

20

TABLE 2: ONCOLOGY

Accession Code	Target Description	Amino Acid	Target Sequence
1 PIP3_HUMAN	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta 3 (EC 3.1.4.11) (Phosphoinositide phospholipase C) (PLC-beta-3) (Phospholipase C-beta-3). 26S proteasome non-ATPase regulatory subunit 1 (26S proteasome regulatory subunit S1) (26S proteasome subunit p112). 26S proteasome non-ATPase regulatory subunit 1 (26S proteasome regulatory subunit S1) (26S proteasome subunit p112). 28S ribosomal protein S31, mitochondrial precursor (S31mt) (MRP-S31) (Imogen 38). 3-phosphoinositide dependent protein kinase-1 (EC 2.7.1.37) (hPDK1). 40S ribosomal protein S3. 52 kDa repressor of the inhibitor of the protein kinase (p58IPK- interacting protein) (58 kDa interferon-induced protein kinase- interacting protein) (P52rIPK) (Death associated protein 4). 53 kDa BRG1-associated factor A (Actin-related protein Baf53a) (ArpNbeta). 5'-AMP-activated protein kinase, catalytic alpha-1 chain (EC 2.7.1.-) (AMPK alpha-1 chain). 5'-AMP-activated protein kinase, catalytic alpha-2 chain (EC 2.7.1.-) (AMPK alpha-2 chain). 60S acidic ribosomal protein P0 (L10E). 60S ribosomal protein L10 (QM protein) (Tumor suppressor QM) (Laminin receptor homolog). 80 kda MCM3-associated protein (GANP protein). Achaete-scute homolog 3 (bHLH transcriptional regulator Sgn-1) (Mash- 3). Achaete-scute homolog 3 (bHLH transcriptional regulator Sgn-1). ADAM 10 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase domain 10) (Mammalian disintegrin-metalloprotease) (Kuzbanian protein homolog).	777 DEEPFD <u>FPK</u> VVLPTL	
2 PSD1_HUMAN		767 TQFWFW <u>FPL</u> SHFLSL	
3 PSD1_HUMAN		807 KPSTFAYPAPLEVPK	
4 RT31_HUMAN		307 EGKLW <u>EF</u> PINNEAGF	
5 PDPK_HUMAN		295 IKLEYD <u>FE</u> PEKFFPKA	
6 RS3_HUMAN		73 VQKR <u>FG</u> PEGSVELY	
7 P52K_HUMAN	18 DLAF <u>FR</u> FRDPARCQ 283 PTVH <u>YE</u> FPNGYNCDF 273 DLPKYL <u>FP</u> EDPSYSS 271 DLPSYL <u>FP</u> EDPSYDA 253 VETDYT <u>FE</u> PLAEKVKA 152 RRAK <u>FK</u> FPGRQKIHI 184 GLTP <u>FS</u> FPQVTNSSV 69 DPY <u>FP</u> FPMPYTNYS 69 EPC <u>FS</u> FPMPYPNYR	18 DLAF <u>FR</u> FRDPARCQ	
8 B53A_HUMAN		283 PTVH <u>YE</u> FPNGYNCDF	
9 AAK1_HUMAN		273 DLPKYL <u>FP</u> EDPSYSS	
10 AAK2_HUMAN		271 DLPSYL <u>FP</u> EDPSYDA	
11 RLA0_HUMAN		253 VETDYT <u>FE</u> PLAEKVKA	
12 RL10_HUMAN		152 RRAK <u>FK</u> FPGRQKIHI	
13 MC3A_MOUSE	69 DPY <u>FP</u> FPMPYTNYS 69 EPC <u>FS</u> FPMPYPNYR  286 PTN <u>PF</u> FRFPNIGVEKF	184 GLTP <u>FS</u> FPQVTNSSV	
14 ASH3_MOUSE		69 DPY <u>FP</u> FPMPYTNYS	
15 ASH3_HUMAN		69 EPC <u>FS</u> FPMPYPNYR	
16 AD10_HUMAN		286 PTN <u>PF</u> FRFPNIGVEKF	
17 AD12_HUMAN		382 ASTGY <u>FP</u> FMVFSSCS	
18 BS69_HUMAN		229 PDNW <u>FC</u> YPCIPNHEL	
19 CYA4_HUMAN	679 ITS <u>LFFF</u> PTSSDCPF 381 EPSK <u>FP</u> FTKDSQHV  661 EVCQ <u>FS</u> YPQTPASPQ	679 ITS <u>LFFF</u> PTSSDCPF	
20 AF4_HUMAN		381 EPSK <u>FP</u> FTKDSQHV	
21 AK11_HUMAN		661 EVCQ <u>FS</u> YPQTPASPQ	
22 AKA3_HUMAN		490 SDIS <u>FE</u> YPEDIGNLS	
23 ALK_HUMAN		264 LECSF <u>DF</u> PCELEYSP	
24 ANR5_HUMAN		437 VIPEYA <u>FP</u> RRRQDGGP	
25 ATR_HUMAN	421 PEQEY <u>FE</u> PEPRNLNN		

26	KRAA_HUMAN	A-Raf proto-oncogene serine/threonine-protein kinase (EC 2.7.1.-) (A-raf-1) (Proto-oncogene Pks). ATP-binding cassette, sub-family A, member 3 (ATP-binding cassette transporter 3) (ATP-binding cassette 3) (ABC-C transporter).	193 DPEHFPFPAPANAPL
27	ABC3_HUMAN	ATP-dependent DNA helicase II, 70 kDa subunit (Lupus Ku autoantigen protein p70) (Ku70) (70 kDa subunit of Ku antigen) (Thyroid-lupus autoantigen) (TLAA) (CTC box binding factor 75 kDa subunit) (CTCBF) (CTC75).	65 LPLFFTFPPPGDTWE
28	KU70_HUMAN	AXIN1 up-regulated gene 1 protein (TGF-beta induced apoptosis protein 3) (TAIP-3) (URAX1 protein).	362 RPSLFVYPEESLVIG
29	AXU1_HUMAN	12751139 B aggressive lymphoma long isoform [Homo sapiens]	88 GITVFYFPRCQGFTS
30		11544936 bA271B5.1 (similar to ribosomal protein S7) [Homo sapiens]	240 SSGIFQFPLNLCTKT
31	ACTY_HUMAN	Beta-centractin (Actin-related protein 1B) (ARP1B).	183 KDVNFEFPEFQLQTK
32	BRC1_HUMAN	Breast cancer type 1 susceptibility protein.	29 QIPKYCFPNYVGRPK
33		Bromodomain adjacent to zinc finger domain protein 1A (ATP-utilizing chromatin assembly and remodeling factor 1) (hACF1) (ATP-dependent chromatin remodelling protein) (Williams syndrome transcription factor-related chromatin remodeling factor 180) (WCRF18	825 DTEGFKYPLGHEVNH
34	BA1A_HUMAN	calcium and DAG-regulated guanine nucleotide exchange factor II [Homo sapiens]	254 QDFSYYFFDDDPPTFI
35		Calcium/calmodulin-dependent protein kinase type II alpha chain (EC 2.7.1.123) (CaM-kinase II alpha chain) (CaM kinase II alpha subunit) (CaMK-II alpha subunit).	627 EEGPFTFPNGEAVEH
36	KCCA_HUMAN	CASP8 and FADD-like apoptosis regulator precursor (Cellular FLICE-like inhibitory protein) (c-FLIP) (Caspase-eight-related protein) (Casper) (Caspase-like apoptosis regulatory protein) (CLARP) (MACH-related inducer of toxicity) (MRIT) (Caspase homolog) (C	226 KAGAYDFPSPWDTV
37	CFLA_MOUSE	Caspase recruitment domain protein 10 (CARD-containing MAGUK protein 3) (Carma 3).	282 HIQLFLFPKSHDITQ
38	CARA_HUMAN	Caspase recruitment domain protein 11 (CARD-containing MAGUK protein 3) (Carma 1).	98 EALEFYYPEHFTLLT
39	CARB_HUMAN	Caspase recruitment domain protein 15 (Nod2 protein) (Inflammatory bowel disease protein 1).	86 ESLEFYYPELYKLVT
40	CARF_HUMAN	Caspase recruitment domain protein 6.	323 QEFLFVFPFSCRQLQ
41	CAR6_HUMAN	Catenin delta-1 (p120 catenin) (p120(ctn)) (Cadherin-associated Src substrate) (CAS) (p120(cas)).	728 LENSWLFPTRIGNF
42	CTD1_HUMAN	Catenin delta-2 (Delta-catenin) (Neural plakophilin-related ARM-repeat protein) (NPRAP) (Neurojungin) (GT24).	202 LPRNFHYPPDGYSRH
43	CTD2_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	162 PEGSFQYPASYHSNQ
44	CEBA_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	27 SSAAFGFPRGAGPAQ
45	CEBA_HUMAN	CCAAT/enhancer binding protein epsilon (C/EBP epsilon).	102 GGGDFDYPGAPAGPG
46	CEBE_HUMAN	Cdc42 GTPase-activating protein [Mus musculus]	84 DPRPFAYPPHTFGPD
47		Cdc42 guanine nucleotide exchange factor zizimin 1.	673 SPAPFPFPEAPGSLP
48	ZIZ1_HUMAN	Cdk5 and abl enzyme substrate 2 (Interactor with cdk3 2) (Ik3-2).	1457 RSLIYKFPSTFYEGR
49	CBL2_HUMAN		241 SYAKFLYPTNALVTH

50 A2M1_HUMAN	Clathrin coat assembly protein AP50 (Clathrin coat associated protein AP50) (Plasma membrane adaptor AP-2 50 kDa protein) (HA2 50 kDa subunit) (Clathrin assembly protein complex 2 medium chain) (AP-2 mu 2 chain).	114 EILDFGYPQNSETGA
51 CTA3_HUMAN	Contactin associated protein-like 3 precursor (Cell recognition molecule Caspr3).	126 EESIWGFPGNTNADS
52 CRKL_HUMAN	Crk-like protein.	128 VRTL_YDFPGNDAEDL
53 CRN1_HUMAN	Crooked neck-like protein 1 (Crooked neck homolog) (hCrm) (CGI-201) (MSTP021).	800 EYFDYIFPEDAANQP
54 11385644	CTCL tumor antigen se2-1 [Homo sapiens]	42 TEDDFEFPFAKTNLS
55 SRA4_RAT	CTD-binding SR-like protein RA4 (Fragment).	158 PQAPFGYPGDGMQQP
56 CG1C_HUMAN	Cyclin C.	138 TRFSYAFPKEFPYRM
57 CCT1_HUMAN	Cyclin T1 (Cyclin T) (CycT1).	598 SSLNFSFPSLPTMGQ
58 CPAC_MOUSE	Cytochrome P450 2A12 (EC 1.14.14.1) (CYP1A12) (Steroid hormones 7- alpha-hydroxylase) (Testosterone 7-alpha-hydroxylase).	456 QNFRFKFPRKLEDIN
59 BMX_HUMAN	Cytoplasmic tyrosine-protein kinase BMX (EC 2.7.1.112) (Bone marrow kinase BMX) (Epithelial and endothelial tyrosine kinase) (ETK) (NTK38).	273 SKISWEFESSSEE
60 PA24_HUMAN	Cytosolic phospholipase A2 (CPLA2) [Includes: Phospholipase A2 (EC 3.1.1.4) (Phosphatidylcholine 2-acylhydrolase); Lysophospholipase (EC 3.1.1.5)].	679 STFNFAQYPNQAFKRL
61 5NTC_HUMAN	Cytosolic purine 5'-nucleotidase (EC 3.1.3.5) (5'-nucleotidase cytosolic II).	260 MTYLFDFPHGPKPGS
62 1616601	disintegrin-metalloprotease MADM [Homo sapiens]	229 PTNPFERFPNISVEKF
63 DM3A_HUMAN	DNA (cytosine-5)-methyltransferase 3A (EC 2.1.1.37) (Dnmt3a) (DNA methyltransferase HsaIIIA) (DNA MTase HsaIIIA) (M.HsaIIIA).	861 MERVFGFPVHYTDVS
64 DM3B_HUMAN	DNA (cytosine-5)-methyltransferase 3B (EC 2.1.1.37) (Dnmt3b) (DNA methyltransferase HsaIIIB) (DNA MTase HsaIIIB) (M.HsaIIIB).	805 LERIFGFPVHYTDVS
65 DNM2_HUMAN	DNA (cytosine-5)-methyltransferase-like protein 2 (Dnmt2) (DNA methyltransferase homolog HsaIIP) (DNA MTase homolog HsaIIP) (M.HsaIIP) (PuMet).	354 FPPEFGFPEKITVKQ
66 MLH3_HUMAN	DNA mismatch repair protein Mlh3 (MutL protein homolog 3).	1285 LGLEFVFVFDTSDSL
67 DPD2_HUMAN	DNA polymerase delta subunit 2 (EC 2.7.7.7).	389 KTDPFIFPECPHVYF
68 DPE2_HUMAN	DNA polymerase epsilon subunit B (EC 2.7.7.7) (DNA polymerase II subunit B).	236 HVNAFGFPPTEPSST
69 DPG2_HUMAN	DNA polymerase gamma subunit 2, mitochondrial precursor (EC 2.7.7.7) (Mitochondrial DNA polymerase accessory subunit) (PolG-beta) (MtPolB) (DNA polymerase gamma accessory 55 kDa subunit) (p55).	287 NKLYYNFPWKGKELIE
70 TP2A_MOUSE	DNA topoisomerase II, alpha isozyme (EC 5.99.1.3).	633 HRIQFKYGPEDDAA
71 TP2B_HUMAN	DNA topoisomerase II, beta isozyme (EC 5.99.1.3).	1444 FGNLFSFPSYSQKSE
72 STAU_HUMAN	Double-stranded RNA-binding protein Staufen homolog.	114 PRYFYFPFPVPLLYQ
73 MPK4_HUMAN	Dual specificity mitogen-activated protein kinase kinase 4 (EC 2.7.1.-) (MAP kinase kinase 4) (JNK activating kinase 1) (c-Jun N- terminal kinase kinase 1) (JNKK) (SAPK/ERK kinase 1) (SEK1).	301 ATGRFPYPKWNSVFD
74 DUS1_HUMAN	Dual specificity protein phosphatase 1 (EC 3.1.3.48) (EC 3.1.3.16) (MAP kinase phosphatase-1) (MKP-1) (Protein-tyrosine phosphatase CL100) (Dual specificity protein phosphatase hVH1).	335 TTTVFNFVPVSVHS

75 DUS4_HUMAN	Dual specificity protein phosphatase 4 (EC 3.1.3.48) (EC 3.1.3.16) (Mitogen-activated protein kinase phosphatase-2) (MAP kinase phosphatase-2) (MKP-2) (Dual specificity protein phosphatase hVH2).	362 SQFVFSFPVSVGVHS
76 EDA_HUMAN	Ectodysplasin A (Ectodermal dysplasia protein) (EDA protein).	134 ALLNFFFPDEKPYSE
77 NPP2_HUMAN	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (E-NPP 2) (Phosphodiesterase I/nucleotide pyrophosphatase 2) (Phosphodiesterase I alpha) (PD-Ialpha) (Autotaxin) [Includes: Alkaline phosphodiesterase I (EC 3.1.4.1); Nucleotide pyrophosphatase (EC 3.6.1.1).	676 MSYGFLFPPYLSSSP
78 EF1G_HUMAN	Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) (PRO1608).	322 WYSEYRFPEELTQTF
79 GAT2_HUMAN	Endothelial transcription factor GATA-2.	167 GSHLFGFPPTPPKEV
80 CASL_HUMAN	Enhancer of filamentation 1 (HEF1) (CRK-associated substrate-related protein) (CAS-L) (CasL) (PP105) (Neural precursor cell expressed developmentally down-regulated 9).	237 REKDYDFPPPMRQAG
81 ESR1_MOUSE	Estrogen receptor (ER) (Estradiol receptor) (ER-alpha).	48 KPTVFNYPEGAAYEF
82 ETV2_HUMAN	Ets translocation variant 2 (Ets-related protein 71).	25 AKLGFCFPDLALQGD
83 ELK3_HUMAN	ETS-domain protein ELK-3 (ETS-related protein NET) (ETS-related protein ERP) (SRF accessory protein 2) (SAP-2).	370 PSTLFQFPPTLLNGHM
84 ELK4_HUMAN	ETS-domain protein ELK-4 (Serum response factor accessory protein 1) (SAP-1).	394 ANTLFQFPVSVLNSHG
85 ERF_HUMAN	ETS-domain transcription factor ERF (Ets2 repressor factor).	138 GGSFHRFPSTPSEV
86 ERF_HUMAN	ETS-domain transcription factor ERF (Ets2 repressor factor).	5 ADTGFAFPDWAYKPE
87 ETV3_HUMAN	ETS-related protein PE-1 (ETS translocation variant 3) (Fragment).	168 ASSRFHFPPLDTHSP
88 ETV3_HUMAN	ETS-related protein PE-1 (ETS translocation variant 3) (Fragment).	35 GGGYQFPDWAYKTE
89 IF33_HUMAN	Eukaryotic translation initiation factor 3 subunit 3 (eIF-3 gamma) (eIF3 p40 subunit) (eIF3h).	78 ITNCFPFPQHTEDDA
90 IF37_HUMAN	Eukaryotic translation initiation factor 3 subunit 7 (eIF-3 zeta) (eIF3 p66) (eIF3d).	331 GKERYNFPNPNPFVE
91 FOL1_HUMAN	Folate receptor alpha precursor (FR-alpha) (Folate receptor 1) (Folate receptor, adult) (Adult folate-binding protein) (FBP) (Ovarian tumor-associated antigen MOV18) (KB cells FBP).	176 QPFHFYFPTPTVLCN
92 FXJ2_HUMAN	Forkhead box protein J2 (Fork head homologous X).	404 NNTGEAFPSDWCSNI
93 FXK1_MOUSE	Forkhead box protein K1 (Myocyte nuclear factor) (MNF).	170 QQCTFRFPSTAIKIQ
94 FZD4_HUMAN	Frizzled 4 precursor (Frizzled-4) (Fz-4) (hFz4) (FzE4).	244 DSSRFSYPERPIIFL
95 GCP6_HUMAN	Gamma-tubulin complex component 6 (GCP-6).	1451 LPRAFAFPVDPQVQS
96 GGPP_HUMAN	Geranylgeranyl pyrophosphate synthetase (GGPP synthetase) (GGPPSASE) (Geranylgeranyl diphosphate synthase) [Includes: Dimethylallyltransferase (EC 2.5.1.1); Geranyltransferase (EC 2.5.1.10); Farnesyltransferase (EC 2.5.1.29)].	209 TEGKFSFPTIHAWS
97 KG3A_HUMAN	Glycogen synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	350 NYTEFKFPQIKAHPW
98 GDF3_HUMAN	Growth/differentiation factor 3 precursor (GDF-3).	88 DQGFFLYPKKISQAS
99 4206785	guanine nucleotide-binding protein [Mus musculus]	104 NLPNFDFFPEFYEHA
100 GBAS_HUMAN	Guanine nucleotide-binding protein G(S), alpha subunit (Adenylate cyclase-stimulating G alpha protein).	136 NVPDFDFPPEFYEHA
101 HM21_HUMAN	High-mobility group protein 2-like 1 (HMGBCG protein).	207 DEESFQYPSQQATVK
102 4151328	high-risk human papilloma viruses E6 oncoproteins targeted protein E6TP1 alpha; putative GAP protein alpha [Homo sapiens]	1054 VSYEFKFPFRNNKW

103 HDA3_HUMAN	Histone deacetylase 3 (HD3) (RPD3-2).	194 KYGN <del>Y</del> FFP <u>GT</u> GDMYE
104 DBX1_MOUSE	Homeobox protein DBX1.	133 PPKTFAFPYFEGSFQ
105 HXCC_HUMAN	Homeobox protein Hox-C12 (Hox-3F).	20 TGDTFYFPNFRASGA
106 HMPH_HUMAN	Homeobox protein PRH (Hematopoietically expressed homeobox) (Homeobox protein HEX).	97 GGPLYPPFPRTVNDYT
107 HIK2_HUMAN	Homeodomain-interacting protein kinase 2 (EC 2.7.1.-).	1067 AQAPYSFPHNSPSHG
108 IKAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150).	599 FVRFPPY <u>PCTQ</u> TELA
109 IP3L_HUMAN	Inositol-trisphosphate 3-kinase B (EC 2.7.1.127) (Inositol 1,4,5- trisphosphate 3-kinase) (IP3K) (IP3 3-kinase) (IP3K-B).	52 RGASFLFP <u>PAES</u> LSP
110 ITA5_HUMAN	Integrin alpha-5 precursor (Fibronectin receptor alpha subunit) (Integrin alpha-F) (VLA-5) (CD49e). Interstitial collagenase precursor (EC 3.4.24.7) (Matrix metalloproteinase-1) (MMP-1) (Fibroblast collagenase).	422 QGVVFVFP <u>GPGG</u> LG
111 MM01_HUMAN	kinesin-related protein HASH [Mus musculus]	364 IYSSFGFPRTVKHID
112 13383464	Kruppel-like factor 4 (Gut enriched kruppel-like factor) (Epithelial zinc-finger protein EZF).	46 NTRDFMFPGPNQMSG
113 KLF4_MOUSE	LAF-4 protein (Lymphoid nuclear protein related to AF4).	134 STCSFSYPIRAGDP
114 LAF4_HUMAN	Lamin A/C (70 kDa lamin).	325 EPTKFFFPNKDSQLV
115 LAMA_HUMAN	large tumor suppressor 1 [Homo sapiens]	477 PLLTYRFPKFTLKA
116 4324434	LAR-interacting protein 1a [Homo sapiens]	334 SSSKFNFPSGRPGMQ
117 930341	Lysophosphatidic acid receptor Edg-2 (LPA receptor 1) (LPA-1).	530 SVPDFRFP <u>MADG</u> HTD
118 EDG2_HUMAN	Lysophosphatidic acid receptor Edg-7 (LPA receptor 3) (LPA-3).	76 VNRRFHFPIYLMAN
119 EDG7_HUMAN	Macrophage metalloelastase precursor (EC 3.4.24.65) (HME) (Matrix metalloproteinase-12) (MMP-12) (Macrophage elastase) (ME).	57 KNRKFHFPPFYLLAN
120 MM12_HUMAN	MAP kinase-activated protein kinase 2 (EC 2.7.1.-) (MAPK-activated protein kinase 2) (MAPKAP kinase 2) (MAPKAP-2).	367 SIHSFGFPNFVKKID
121 MKK2_HUMAN	Melanoma antigen preferentially expressed in tumors (Preferentially expressed antigen of melanoma) (OPA-interacting protein 4) (OIP4).	280 RMGQYEF <u>PNPEW</u> SEV
122 MAPE_HUMAN	Melanoma-associated antigen 3 (MAGE-3 antigen) (Antigen MZ2-D).	140 RASLYSFPEPEAAQP
123 MAG3_HUMAN	Metalloproteinase inhibitor 3 precursor (TIMP-3) (Tissue inhibitor of metalloproteinases-3) (MIG-5 protein).	141 GNWQYFFFPVIFSKAS
124 TIM3_HUMAN	Methionine aminopeptidase 1 (EC 3.4.11.18) (MetAP 1) (Peptidase M 1) (Fragment).	168 MLSNFGYPGYQSKHY
125 AMP1_HUMAN	Microtubule-associated protein 1A (MAP 1A) (Proliferation-related protein p80) [Contains: MAP1 light chain LC2].	191 PLNYNFPKSCCTSV
126 MAPA_HUMAN	Mitochondrial 28S ribosomal protein S29 (S29mt) (MRP-S29) (Death- associated protein 3) (DAP-3) (Ionizing radiation resistance conferring protein).	37 KPCCYIFPGGRGDSA
127 RT29_HUMAN	Mitogen activated protein kinase activated protein kinase [Homo sapiens]	113 KNTSFAYPAIRYLLY
128 3133291	Mitogen-activated protein kinase 6 (EC 2.7.1.-) (Extracellular signal- regulated kinase 3) (ERK-3)	259 MTGSFEFPEEWSQI
129 MK06_HUMAN	(MAP kinase isoform p97) (p97-MAPK).	315 YMSIYSFPMDEPISS
130 M3K6_HUMAN	Mitogen-activated protein kinase kinase 6 (EC 2.7.1.-).	800 HRPLFAFPD <u>AVKQ</u> IL

131	TAB1_HUMAN	Mitogen-activated protein kinase kinase kinase 7 interacting protein 1 (TAK1-binding protein 1). Mitogen-activated protein kinase kinase kinase 2 (EC 2.7.1.37) (MAPK/ERK kinase kinase kinase 2) (MEK kinase 2) (MEKKK 2) (Germinal center kinase) (GC kinase) (Rab8 interacting protein) (B lymphocyte serine/threonine protein kinase).	361	LVRNFGYPLGEMSQP
132	M4K2_HUMAN	Multidrug resistance protein 1 (P-glycoprotein 1) (CD243 antigen).	737	PELTDFPIETVCL
133	MDR1_HUMAN	Multidrug resistance protein 1 (P-glycoprotein 1) (CD243 antigen).	1038	GEVFNYPTRPDIPV
134	MDR1_HUMAN	Multidrug resistance protein 3 (P-glycoprotein 3).	395	RNVHFSYPSRKEVKI
135	MDR3_HUMAN	Multidrug resistance protein 3 (P-glycoprotein 3).	88	TAGNFSFPVNFSLSL
136	MDR3_HUMAN	Multidrug resistance protein 3 (P-glycoprotein 3).	397	NDVHFSYPSRANVKI
137	DRNL_HUMAN	Muscle-specific DNase I-like precursor (EC 3.1.21.-) (DNase X) (XIB).	242	TAAAFDFPTSFQLTE
138	6959304	MYB-binding protein 1A [Homo sapiens]	497	TKHPFSFPLENQARE
139	2706549	myc-intron-binding protein-1 [Mus musculus]	135	SEDLFPFPMHGHSGG
140	MY15_HUMAN	Myosin XV (Unconventional myosin-15).	751	RGAAFGFPGASPRAS
141	MM08_HUMAN	Neutrophil collagenase precursor (EC 3.4.24.34) (Matrix metalloproteinase-8) (MMP-8) (PMNL collagenase) (PMNL-CL).	364	DISNYGFSPSSVQAID
142	KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP- 1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	405	SFPHYGFPTYGGITF
143	KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP- 1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	400	TGPGYSFPHYGFPTY
144	RI14_HUMAN	Nuclear factor RIP140 (Nuclear receptor interacting protein 1).	996	DNRTFSYPGVVKTPV
145	RI14_HUMAN	Nuclear factor RIP140 (Nuclear receptor interacting protein 1).	905	NDLEFKYPAGHGSAS
146	N153_HUMAN	Nuclear pore complex protein Nup153 (Nucleoporin Nup153) (153 kDa nucleoporin).	421	RESGFSYPNFSLPAA
147	NCR1_HUMAN	Nuclear receptor co-repressor 1 (N-CoR1) (N-CoR).	23	HSVQYTFPNTRHQQE
148	RORG_HUMAN	Nuclear receptor ROR-gamma (Nuclear receptor RZR-gamma).	540	PPSPFSFPMNPGGWS
149	DD21_HUMAN	Nucleolar RNA helicase II (Nucleolar RNA helicase Gu) (RH II/Gu) (DEAD-box protein 21).	136	RGVTFLFPIQAKTFH
150	NDK5_HUMAN	Nucleoside diphosphate kinase homolog 5 (NDK-H 5) (NDP kinase homolog 5) (nm23-H5) (Testis-specific nm23 homolog) (Inhibitor of p53-induced apoptosis-beta) (PIA-beta).	137	REIRFMFPEVIVEPI
151	OXE2_HUMAN	Olfactory receptor 51E2 (Prostate specific G-protein coupled receptor) (HPRAJ).	150	RGSLFFFFPLPLLIKR
152	ORC4_HUMAN	Origin recognition complex subunit 4.	217	LMNSFGFPQYVKIFK
153	ORC5_HUMAN	Origin recognition complex subunit 5.	24	ERHHFSFPSIFIYGH
154	2645205	p160 myb-binding protein [Mus musculus]	495	TKQHFSFPLDDRNRG
155	PAX1_HUMAN	Paxillin.	114	EEHVYSFPPNKQKSAE
156	CYP6_HUMAN	Peptidyl-prolyl cis-trans isomerase like 2 (EC 5.2.1.8) (PPlase) (Rotamase) (Cyclophilin-60) (Cyclophilin-like protein Cyp-60).	45	SLQPFVYPVCTPDGI
157	CSKP_HUMAN	Peripheral plasma membrane protein CASK (EC 2.7.1.-) (hCASK) (Calcium/calmodulin-dependent serine protein kinase) (Lin-2 homolog).	763	HPDRFAYPIPHTRRP
158	PEC1_HUMAN	Peroxisomal 3,2-trans-enoyl-CoA isomerase (EC 5.3.3.8) (Dodecenoyl-CoA delta-isomerase) (D3,D2-enoyl-CoA isomerase) (DBI-related protein 1) (DRS-1) (Hepatocellular carcinoma-associated antigen 88).	246	GCCSYTFPKIMSPAK

159	PPAR_HUMAN	Peroxisome proliferator activated receptor alpha (PPAR-alpha).	417	PDDIFLFPKLLQKMA
160	PPAS_MOUSE	Peroxisome proliferator activated receptor delta (PPAR-delta) (PPAR-beta) (Nuclear hormone receptor 1) (NUC1).	389	PDSQYLFPKLLQKMA
161	P85A_MOUSE	Phosphatidylinositol 3-kinase regulatory alpha subunit (PI3-kinase p85-alpha subunit) (PtdIns-3-kinase p85-alpha) (PI3K).	269	SPVLFRRFPAASSDNT
162	PI4K_HUMAN	Phosphatidylinositol 4-kinase alpha (EC 2.7.1.67) (PI4-kinase) (PtdIns-4-kinase) (PI4K-alpha).	628	GLDLFVFPYRVVATA
163	PTEN_HUMAN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase PTEN (EC 3.1.3.67) (Mutated in multiple advanced cancers 1).	237	KFMYFEFPQPLPVC
164	P11B_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, beta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit beta) (PtdIns-3-kinase p110) (PI3K) (PI3Kbeta).	608	ELLDNFNYPDQYVREY
165	P11D_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) (PtdIns-3-kinase p110) (PI3K) (p110delta).	581	ELLDFSFPDCHVGSF
166	P11D_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) (PtdIns-3-kinase p110) (PI3K) (p110delta).	161	AWLQYSFPLQLEPSA
167	940231	phosphodiesterase A' subunit [Homo sapiens]	366	ADEYFTFPKGPVDET
168	22758919	phosphoinositide 3 kinase P110delta [Mus musculus]	580	ELLDFSFPDCYVGSF
169	2696236	phospholipase B [Rattus norvegicus]	42	TLKNFSFPCKPKKLE
170	PLEK_HUMAN	Pleckstrin (Platelet p47 protein).	213	PDAFYFPPDSGFFCE
171	3002588	Plenty of SH3s; POSH [Mus musculus]	376	TGPAFTFPSPDVPYQA
172	PAB5_HUMAN	Polydenylate-binding protein 5 (Poly(A)-binding protein 5) (PABP 5).	176	YVGRFKFPEERAAEV
173	PAB5_HUMAN	Polydenylate-binding protein 5 (Poly(A)-binding protein 5) (PABP 5).	62	GYVNFRFPADAEWAL
174	15667468	polyploidy associated protein kinase PAK-A [Mus musculus]	402	SELEFQFPDDKDPVW
175	DP1_HUMAN	Polyposis locus protein 1 (TB2 protein).	56	NLIGFGYPAYISKA
176	CIQ3_HUMAN	Potassium voltage-gated channel subfamily KQT member 3 (Potassium channel KQT-like 3).	587	KGSAFTFPSQQSPRN
177	MV10_HUMAN	Potentail helicase MOV-10.	601	KKGEYVFPACKKLE
178	A11A_HUMAN	Potential phospholipid-transporting ATPase IS (EC 3.6.3.1) (Fragment).	552	KNVCFIFPQFLYQFF
179	4097902	potential transcriptional repressor Not4hp [Mus musculus]	485	VYNSFGFPQAARYP
180	PRD2_HUMAN	PR-domain zinc finger protein 2 (Retinoblastoma protein-interacting zinc-finger protein) (MTE-binding protein) (MTB-ZF).	354	VFETFMFPCQHCEK
181	DDX6_HUMAN	Probable ATP-dependent RNA helicase p54 (Oncogene RCK) (DEAD-box protein 6).	405	VVINDFFPKLAETYL
182	GPR4_HUMAN	Probable G protein-coupled receptor GPR4 (GPR19).	186	VFVGFLFPWALMLLS
183	GP68_HUMAN	Probable G protein-coupled receptor GPR68 (Ovarian cancer G protein- coupled receptor 1) (OGR-1).	190	FLVGFLFPICLLLAS
184	K682_HUMAN	Probable RNA-binding protein KIAA0682.	446	AFITFMFPEHAVKAY
185	MN1_HUMAN	Probable tumor suppressor protein MN1.	455	QEARFDFPGSAGVDR
186	FAFX_HUMAN	Probable ubiquitin carboxyl-terminal hydrolase FAF-X (EC 3.1.2.15) (Ubiquitin thiolesterase FAF-X) (Ubiquitin-specific processing protease FAF-X) (Deubiquitinating enzyme FAF-X) (Fat facets protein related, X-linked) (Ubiquitin-specific protease 9, X chro	1805	FNDYFEFPRELDMEP

187 FAFY_HUMAN	Probable ubiquitin carboxyl-terminal hydrolase FAF-Y (EC 3.1.2.15) (Ubiquitin thiolesterase FAF-Y)
188 PDC2_HUMAN	(Ubiquitin-specific processing protease FAF-Y) (Deubiquitinating enzyme FAF-Y) (Fat facets protein related, Y-linked) (Ubiquitin-specific protease 9, Y chro
189 3858885	Programmed cell death protein 2 (Zinc finger protein Rp-8).
190 KPCM_HUMAN	proliferation potential-related protein [Mus musculus]
191 KPCN_HUMAN	Protein kinase C, mu type (EC 2.7.1.-) (nPKC-mu) (Protein kinase D).
192 15080775	Protein kinase C, nu type (EC 2.7.1.-) (nPKC-nu) (Protein kinase EPK2).
193 PP3D_HUMAN	protein kinase NYD-SP5 [Homo sapiens]
194 10567793	Protein phosphatase 1, regulatory subunit 3D (Protein phosphatase 1, regulatory subunit 6) (Protein phosphatase 1 binding subunit R6).
195 1144002	protein tyrosine phosphatase BK [Mus musculus]
196 23268287	protein tyrosine phosphatase D30 [Rattus norvegicus]
197 PTND_HUMAN	protein tyrosine phosphatase receptor-like protein J [Mus musculus]
198 PAR2_HUMAN	Protein tyrosine phosphatase, non-receptor type 13 (EC 3.1.3.48) (Protein-tyrosine phosphatase 1E) (PTP-E1) (hPTE1) (PTP-BAS) (Protein-tyrosine phosphatase PTPL1) (Fas-associated protein-tyrosine phosphatase 1) (FAP-1).
199 PTPJ_MOUSE	Proteinase activated receptor 2 precursor (PAR-2) (Thrombin receptor-like 1) (Coagulation factor II receptor-like 1).
200 PTPZ_RAT	Protein-tyrosine phosphatase eta precursor (EC 3.1.3.48) (R-PTP-eta) (HPTP beta-like tyrosine phosphatase).
201 PTPZ_RAT	Protein-tyrosine phosphatase zeta precursor (EC 3.1.3.48) (R-PTP- zeta) (Phosphacan) (3F8 chondroitin sulfate proteoglycan) (3H1 keratan sulfate proteoglycan).
202 FAT2_HUMAN	Protein-tyrosine phosphatase zeta precursor (EC 3.1.3.48) (R-PTP- zeta) (Phosphacan) (3F8 chondroitin sulfate proteoglycan) (3H1 keratan sulfate proteoglycan).
203 8216989	Protocadherin Fat 2 precursor (hFat2) (Multiple epidermal growth factor-like domains 1).
204 GP40_HUMAN	putative cell cycle control protein [Homo sapiens]
205 RBM9_HUMAN	Putative G protein-coupled receptor GPR40.
206 6007826	Putative RNA-binding protein 9 (RNA binding motif protein 9).
207 RAE1_HUMAN	rab escort protein-2 [Mus musculus]
208 RAE2_HUMAN	Rab proteins geranylgeranyltransferase component A 1 (Rab escort protein 1) (REP-1)
209 RBPL_HUMAN	(Choroideraemia protein) (TCD protein).
210 RB_HUMAN	Rab proteins geranylgeranyltransferase component A 2 (Rab escort protein 2) (REP-2)
211 RBB5_HUMAN	(Choroideraemia-like protein).
212 RBB8_HUMAN	Recombining binding protein suppressor of hairless-like protein (Transcription factor RBP-L).
	Retinoblastoma-associated protein (PP110) (P105-RB) (RB).
	Retinoblastoma-binding protein 5 (RBBP-5) (Retinoblastoma-binding protein RBQ-3).
	Retinoblastoma-binding protein 8 (RBBP-8) (CtBP interacting protein) (CtIP) (Retinoblastoma-interacting protein and myosin-like) (RIM).

1616 RDDVFGYPHQFEDKP  
183 PDHNFLFPEFEIVIE  
742 TDTLFFVPSREDATP  
794 QNAAFMYPPNPWKEI  
787 QNAAFMYPPNPWREI  
97 DQKSFIFPQESEGTF

234 DVFTFGFPVPPFLLLE  
262 RDRRFHFPEETPETP  
261 RDRPFHFPEETPETP  
172 TNSFAFPESNETQA

1704 ICTMFYYPQKIPNKP

247 AIGVFLFPAFLTASA

172 TNNTFAFPESNETQA

1565 TSTDSEFPDVNEKDA

720 HYSTFAYPPTTEVTSH  
4222 YGGFPPFPLEMENKR  
100 NNQLFRFPATSPLKT  
244 NVASFLYPNLGGSWR  
251 IPGFPYPTAATTAA  
370 GNTPFIFPLYGHGEI

369 GNTPFLLPLYGQGEI

371 GNTPFLLPLYGQGEI  
311 HKCAFQFPGSPGGG  
786 PRSPYKFPSSPLRIP  
100 CDQRFRRFPSPILKVQ

467 KENAFPPMDNQFSM

213 RBL2_HUMAN	Retinoblastoma-like protein 2 (130 kDa retinoblastoma-associated protein) (PRB2) (P130) (RBR-2).	170 FQDI <b>F</b> KYPQEEQPRQ
214 12053793	retinoid-acid induced protein 1 [Homo sapiens]	1830 PISL <b>S</b> FSFP <b>L</b> LPQQF
215 ARH2_HUMAN	Rho guanine nucleotide exchange factor 2 (GEF-H1 protein) (Proliferating cell nucleolar antigen p40).	505 KDQKYI <b>F</b> PTLDKPSV
216 RHG7_HUMAN	Rho-GTPase-activating protein 7 (Rho-type GTPase-activating protein 7) (Deleted in liver cancer 1 protein) (Dlc-1) (HP protein) (StAR-related lipid transfer protein 12) (StARD12) (START domain-containing protein 12).	34 LYED <b>F</b> L <b>F</b> IDISLVK
217 RBMS_HUMAN	RNA-binding protein with multiple splicing (RBP-MS).	163 PPPA <b>F</b> TYPASLHAQM
218 RUN2_HUMAN	Run1-related transcription factor 2 (Core-binding factor, alpha 1 subunit) (CBF-alpha 1) (Acute myeloid leukemia 3 protein) (Oncogene AML-3) (Polyomavirus enhancer binding protein 2 alpha A subunit) (PEBP2-alpha A) (PEA2-alpha A) (SL3-3 enhancer factor 1	450 SSGSYQ <b>F</b> PMVPGGDR
219 SEN2_HUMAN	Sentrin-specific protease 2 (EC 3.4.22.-) (Sentrin/SUMO-specific protease SENP2).	66 AASL <b>F</b> GG <b>F</b> Q <b>L</b> TTKP
220 SEN2_HUMAN	Sentrin-specific protease 2 (EC 3.4.22.-) (Sentrin/SUMO-specific protease SENP2).	437 VFST <b>F</b> FYP <b>K</b> LKSGGY
221 SEP1_HUMAN	Septin 1 (LARP) (Serologically defined breast cancer antigen NY-BR- 24).	195 EIH <b>I</b> YQ <b>F</b> ECDSDED
222 SEP4_HUMAN	Septin 4 (Peanut-like protein 2) (Brain protein H5) (Cell division control-related protein 2) (hCDCREL-2) (Bradeion beta) (CE5B3 beta) (Cerebral protein-7) (hucep-7).	314 GIK <b>I</b> YQ <b>F</b> DCDSEDED
223 SEP5_HUMAN	Septin 5 (Peanut-like protein 1) (Cell division control related protein 1) (CDCREL-1).	214 GIHV <b>Y</b> Q <b>F</b> ECDSDED
224 SEP7_HUMAN	Septin 7 (CDC10 protein homolog).	200 KIK <b>I</b> Y <b>F</b> EPETDDEEE
225 STK6_HUMAN	Serine/threonine kinase 6 (EC 2.7.1.37) (Serine/threonine kinase 15) (Aurora/PL1-related kinase 1) (Aurora-related kinase 1) (hARK1) (Aurora-A) (Breast-tumor-amplified kinase).	342 SRVE <b>F</b> TFP <b>D</b> FVTEGA
226 STKD_MOUSE	Serine/threonine protein kinase 13 (EC 2.7.1.37) (Aurora/lpl1/Eg2 protein 1).	225 RQVD <b>F</b> K <b>F</b> PSSVPAGA
227 KPT3_HUMAN	Serine/threonine protein kinase PCTAIRE-3 (EC 2.7.1.-).	371 EFRT <b>Y</b> S <b>F</b> PCYLPQPL
228 2ACA_HUMAN	Serine/threonine protein phosphatase 2A, 72/130 kDa regulatory subunit B (PP2A, subunit B, B"-PR72/PR130) (PP2A, subunit B, B72/B130 isoforms) (PP2A; subunit B, PR72/PR130 isoforms)	708 NIP <b>R</b> FY <b>F</b> PEGLPDTC
229 ST19_HUMAN	(PP2A, subunit B, R3 isoform).	96 SLHS <b>Y</b> P <b>F</b> P <b>G</b> TIKSRD
230 MAK_MOUSE	Serine/threonine-protein kinase 19 (EC 2.7.1.37) (RP1 protein) (G11 protein).	432 KES <b>P</b> FR <b>F</b> PD <b>S</b> GLPVS
231 MAK_HUMAN	Serine/threonine-protein kinase MAK (EC 2.7.1.-) (Male germ cell-associated kinase).	232 SSMN <b>F</b> R <b>F</b> PQ <b>C</b> VPINL
232 KPT2_HUMAN	Serine/threonine-protein kinase PCTAIRE-2 (EC 2.7.1.-).	421 EFKN <b>Y</b> N <b>F</b> P <b>K</b> YKPEPL
233 ULK1_HUMAN	Serine/threonine-protein kinase ULK1 (EC 2.7.1.-) (Unc-51-like kinase 1).	627 AVPS <b>F</b> D <b>F</b> P <b>K</b> TPSSQN
234 ST5A_HUMAN	Signal transducer and activator of transcription 5A.	664 SYLI <b>Y</b> V <b>F</b> P <b>D</b> RPKDEV
235 ST5B_HUMAN	Signal transducer and activator of transcription 5B.	664 NYLI <b>Y</b> V <b>F</b> P <b>D</b> RPKDEV
236 CBL_HUMAN	Signal transduction protein CBL (Proto-oncogene c-CBL).	333 REG <b>F</b> YL <b>F</b> P <b>D</b> GRNQNP
237 CBLB_HUMAN	Signal transduction protein CBL-B (SH3-binding protein CBL-B).	966 ILRE <b>F</b> A <b>F</b> PP <b>P</b> VSPRL
238 6649242	splicing coactivator subunit SRm300 [Homo sapiens]	2225 SSS <b>S</b> F <b>F</b> P <b>F</b> CKAWPSG
239 S3B3_HUMAN	Splicing factor 3B subunit 3 (Spliceosome associated protein 130) (SAP 130) (SF3b130) (Pre-mRNA splicing factor SF3b 130 kDa subunit).	1162 SFR <b>S</b> Y <b>F</b> FPVKNVIDG

240 STAC_HUMAN	Stac protein (SRC homology 3 and cysteine-rich domain protein).	253 SNSVETY <u>PENG</u> TDDF
241 TX18_HUMAN	T-box transcription factor TBX18 (T-box protein 18) (Fragment).	186 GVKAFSFPETVFTTV
242 TX20_HUMAN	T-box transcription factor TBX20 (T-box protein 20) (Fragment).	205 EFRTFIFPETVFTAV
243 TBX3_HUMAN	T-box transcription factor TBX3 (T-box protein 3).	597 FGSLEFPYPYTYMAAA
244 TBX6_HUMAN	T-box transcription factor TBX6 (T-box protein 6).	235 GMASFRFPETTFISV
245 T2AY_HUMAN	TFIIA-alpha and beta like factor (ALF).	106 SSANFTFPGYPIHVP
246 TFH3_HUMAN	TFIIH basal transcription factor complex p34 subunit (Basic transcription factor 2 34 kDa subunit) (BTF2-p34) (General transcription factor IIF polypeptide 3).	65 QESRFLYPGKNGRLG
247 TOB1_HUMAN	Tob1 protein (Transducer of erbB-2 1).	270 NAKFIFPNMQGQGS
248 GAT3_HUMAN	Trans-acting T-cell specific transcription factor GATA-3.	147 SPHLFTFPPTPPKDV
249 GAT4_HUMAN	Transcription factor GATA-4 (GATA binding factor-4).	104 VSPRFSFPGTTGSLA
250 GAT5_HUMAN	Transcription factor GATA-5 (GATA binding factor-5).	365 EPEDFAFPSTAPSPQ
251 TF65_MOUSE	Transcription factor p65 (Nuclear factor NF-kappa-B p65 subunit).	345 APQPYTFPASLSTIN
252 TCL5_HUMAN	Transcription factor-like 5 protein (Cha transcription factor) (HPV-16 E2 binding protein 1) (E2BP-1).	203 TALQETYPLFTTNAC
253 TF1A_HUMAN	Transcription intermediary factor 1-alpha (TIF1-alpha) (Tripartite motif protein 24).	729 PPENYDFPVVVKQE
254 ERG_MOUSE	Transcriptional regulator ERG (Fragment).	52 GGAAFIPTNTSVYPE
255 TERA_HUMAN	Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)- ATPase p97 subunit) (Valosin containing protein) (VCP) [Contains: Valosin].	767 GFGSFRFPNGQGA
256 E2BE_HUMAN	Translation initiation factor eIF-2B epsilon subunit (eIF-2B GDP-GTP exchange factor).	220 GLRRFAFPLSLFQGS
257 SSRA_HUMAN	Translocon-associated protein, alpha subunit precursor (TRAP-alpha) (Signal sequence receptor alpha subunit) (SSR-alpha).	120 LDASFRYPQDYQFYI
258 TRIO_HUMAN	Triple functional domain protein (PTPRF interacting protein).	2437 RPSFTFPDSDSLQ
259 1113923	tyrosine phosphatase-like protein IA-2a [Rattus norvegicus]	947 SKDKFEFFLTPVGEE
260 BTK_HUMAN	Tyrosine-protein kinase BTK (EC 2.7.1.112) (Bruton's tyrosine kinase) (Agammaglobulinaemia tyrosine kinase) (ATK) (B cell progenitor kinase) (BPK).	94 IIERFPYPFQVWYDE
261 JAK2_HUMAN	Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).	114 YRIRFYFPRWYCSGS
262 JAK3_HUMAN	Tyrosine-protein kinase JAK3 (EC 2.7.1.112) (Janus kinase 3) (JAK-3) (Leukocyte janus kinase) (L-JAK).	100 YRIRFYFPNWFGLEK
263 KSYK_HUMAN	Tyrosine-protein kinase SYK (EC 2.7.1.112) (Spleen tyrosine kinase).	292 RIKSYSFPKPGHRKS
264 ROR1_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 1).	785 RYPNYMFPSQGITPQ
265 ROR2_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR2 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 2).	230 SFCHFVFPLCDARSR
266 UBPO_MOUSE	Ubiquitin carboxyl-terminal hydrolase 26 (EC 3.1.2.15) (Ubiquitin thiolesterase 26) (Ubiquitin-specific processing protease 26) (Deubiquitinating enzyme 26).	223 SKPGFGFPFETNYPE
267 UBP8_HUMAN	Ubiquitin carboxyl-terminal hydrolase 8 (EC 3.1.2.15) (Ubiquitin thiolesterase 8) (Ubiquitin-specific processing protease 8) (Deubiquitinating enzyme 8).	330 ISLDFTYPSLEESIP
268 UB4B_HUMAN	Ubiquitin conjugation factor E4 B (Ubiquitin-fusion degradation protein 2).	537 DSDYFKYPLMALGEL

269	27434480	ubiquitin ligase E3 alpha-II [Homo sapiens] Ubiquitin--protein ligase EDD (EC 6.3.2.-) (Hyperplastic discs protein homolog) (hHYD) (Progesterin induced protein).	80 <u>EDPAFGFPKLEQANK</u>
270	EDD_HUMAN	Valyl-tRNA synthetase 2 (EC 6.1.1.9) (Valine--tRNA ligase 2) (ValRS 2) (G7a).	274 <u>DISYFGYPSFRRSSL</u>
271	SYV2_HUMAN	Vault poly(ADP-ribose) polymerase (EC 2.4.2.30) (VPARP) (193-kDa vault protein) (PARP-related/lalpal-related H5/proline-rich) (PH5P).	1001 <u>GFQAYDFPAVTTAQY</u>
272	PPOV_HUMAN	Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	921 <u>YKELFSYPKHITSNT</u>
273	VINE_HUMAN	Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).	565 <u>RRTGFSFPTQEPRPQ</u>
274	VINE_HUMAN	Werner syndrome helicase.	352 <u>GRRDFVYPSSTRDPS</u>
275	WRN_HUMAN	Wiskott-Aldrich syndrome protein family member 2 (WASP-family protein member 2) (Verprolin homology domain-containing protein 2).	577 <u>KSLCFQYPPVYVGKI</u>
276	WAS2_HUMAN	Wiskott-Aldrich syndrome protein family member 2 (WASP-family protein member 2) (Verprolin homology domain-containing protein 2).	270 <u>PPAEFSYPVDNQGRGS</u>
277	WAS2_HUMAN	WW domain binding protein 2 (WBP-2).	218 <u>KLGPFGYPPTLVYQN</u>
278	WBP2_MOUSE	Zinc finger protein 44 (Zinc finger protein KOX7) (Gonadotropin inducible transcription repressor-2) (GIOT-2).	149 <u>PSGAYVFPPPVANGM</u>
279	ZN44_HUMAN	Zinc finger protein HRX (ALL-1) (Trithorax-like protein).	342 <u>CGKGFDFPGSARIHE</u>
280	HRX_HUMAN	Zinc finger protein ZNF287.	792 <u>LNPTFTFPSHSLTQS</u>
281	Z287_HUMAN		44 <u>NFRNFPYPDLAGPRK</u>

TABLE 3: CARDIOVASCULAR

Accession Code	Target Description	Amino Acid	Target Sequence
1 ACHB_HUMAN	Acetylcholine receptor protein, beta chain precursor.	395	<u>PPSDFLFKPNRFQP</u>
2 CYA4_HUMAN	Adenylate cyclase, type IV (EC 4.6.1.1) (ATP pyrophosphate-lyase) (Adenylyl cyclase).	679	<u>ITSLFFFTSSDCPF</u>
3 GAT2_HUMAN	Endothelial transcription factor GATA-2.	167	<u>GSHLFGFPPTPPKEV</u>
4 CXA1_HUMAN	Gap junction alpha-1 protein (Connexin 43) (Cx43) (Gap junction 43 kDa heart protein).	330	<u>HAQPFDFPDDNQNSK</u>
5 GBAS_HUMAN	Guanine nucleotide-binding protein G(S), alpha subunit (Adenylate cyclase-stimulating G alpha protein).	136	<u>NVPDFDFPPEFYEHA</u>
6 IKAP_HUMAN	IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150).	599	<u>FPVRFPYPCTQTELA</u>
7 LGR6_HUMAN	Leucine-rich repeat-containing G protein-coupled receptor 6.	751	<u>GLETYGFPSVTLISC</u>
8 MYHD_HUMAN	Myosin heavy chain, skeletal muscle, extraocular (MyHC-eo).	306	<u>STNPFDFPFVSQGEV</u>
9 NDR4_HUMAN	NDRG4 protein (Brain development-related molecule 1) (Vascular smooth muscle cell associated protein-8) (SMAP-8).	80	<u>FPQGYQFPSMEQLAA</u>
10 ACHO_RAT	Neuronal acetylcholine receptor protein, beta-3 chain precursor.	358	<u>PMDRFSFPDGKESDT</u>
11 P2X1_HUMAN	P2X purinoceptor 1 (ATP receptor) (P2X1) (Purinergic receptor).	86	<u>DVADYVFPAAQGDNSF</u>
12 P2X7_HUMAN	P2X purinoceptor 7 (ATP receptor) (P2X7) (Purinergic receptor) (P2Z receptor).	89	<u>DTADYTFPLQGNSTFF</u>
13 GPR4_HUMAN	Probable G protein-coupled receptor GPR4 (GPR19).	186	<u>VFVGFLFPWALMLLS</u>

14 GP68_HUMAN	Probable G protein-coupled receptor GPR68 (Ovarian cancer G protein- coupled receptor 1) (OGR-1).	190 FLVGLF <u>LPICLLAS</u>
15 GP17_HUMAN	Probable P2Y purinoceptor GPR17 (P2Y-like receptor) (R12).	225 LAVAFTFPFITVTC
16 GP40_HUMAN	Putative G protein-coupled receptor GPR40.	244 NVASFLYPNLGGSWR
17 FK79_HUMAN	Putative P2Y purinoceptor FKSG79.	244 APYHFSFPLDFLVKS
18 LGR7_HUMAN	Relaxin receptor 1 (Leucine-rich repeat-containing G protein-coupled receptor 7).	734 KPDLFTYPCEMSLIS
19 AG2R_HUMAN	Type-1 angiotensin II receptor (AT1) (AT1AR).	200 NILGFLFPFLILTS
20 VNN2_HUMAN	Vascular non-inflammatory molecule 2 precursor (Vanin 2) (Glycosylphosphatidyl inositol-anchored protein GPI-80) (FOAP-4 protein).	435 FGTEYVFPEVLLTEI
21 16904210	very large G protein-coupled receptor 1 [Mus musculus]	1971 PYGVFIFPNKTRPLS
22 VWF_HUMAN	Von Willebrand factor precursor (vWF).	879 DGLKYLFPGECCQYVL
23 WS14_HUMAN	Williams-Beuren syndrome chromosome region 14 protein (WS basic-helix- loop-helix leucine zipper protein) (WS-bHLH) (Mlx interactor).	431 FSPRFPFPTVPPAPG

TABLE 4: INFLAMMATION

Accession Code	Target Description	Amino Acid	Target Sequence
1 A1M2_HUMAN	Adaptor-related protein complex 1, mu 2 subunit (Mu-adaptin 2) (Adaptor protein complex AP-1 mu-2 subunit) (Golgi adaptor HA1/AP1 adaptin mu-2 subunit) (Clathrin assembly protein assembly protein complex 1 medium chain 2) (AP-mu chain family member mu1B). Allograft inflammatory factor-1 (AIF-1) (Ionized calcium-binding adapter molecule 1) (G1). Apolipoprotein L3 (Apolipoprotein L-III) (ApoL-III) (TNF-inducible protein CG12-1) (CG12_1). Calcineurin-binding protein Cabin 1 (Calcineurin inhibitor) (CAIN). CCAAT/enhancer binding protein alpha (C/EBP alpha). CCAAT/enhancer binding protein epsilon (C/EBP epsilon) (C/EBP-related protein 1). Complement C4 precursor [Contains: C4A anaphylatoxin]. C-X-C chemokine receptor type 3 (CXC-R3) (CXCR-3) (CKR-L2) (CD183 antigen). Endothelial transcription factor GATA-2. FYN-binding protein (FYN-T-binding protein) (FYB-120/130) (p120/p130) (SLP-76 associated phosphoprotein) (SLAP-130).	116	ELMDFGFPQTDSKI
2 AIF1_HUMAN		97	SGETFSYPDFLRMML
3 APL3_HUMAN		23	QVWTFITFPFGFGGIS
4 CAB1_HUMAN		947	FYCLYSFSPSKKSKAR
5 CEBA_HUMAN		102	GGGDFDYPGAPAGPG
6 CEBE_RAT		84	DPRPFAYPSTHTFGPD
7 CO4_HUMAN		915	ARGSFEFPVGDVSK
8 CCR3_HUMAN		201	THCQYNFPQVGR TAL
9 GAT2_HUMAN		167	GSHLFGFPPTPPKEV
10 FYB_HUMAN	HMG-box protein SOX21 [Mus musculus] IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150). Interferon regulatory factor 4 (IRF-4) (Lymphocyte specific interferon regulatory factor) (LSIRF) (NF-EM5) (Multiple myeloma oncogene 1). Interleukin enhancer-binding factor 1 (Cellular transcription factor ILF-1). Interleukin-1 receptor-associated kinase-2 (EC 2.7.1.-) (IRAK-2). Large proline-rich protein BAT2 (HLA-B-associated transcript 2) (G2).	125	SKPTFPWPPGNKPSL
11 28274770		88	KKDKFAFPVPYGLGS
12 IKAP_HUMAN		599	FPVRFPPYPCQTTELA
13 IRF4_HUMAN		288	DQVLFYPYPEDNGQRK
14 ILF1_HUMAN		134	RVCTFRFPSTNIKIT
15 IRA2_HUMAN		280	QFHSFIYPYMANGSL
16 BAT2_HUMAN		249	MMPPFMYPYPYLPFPP

17 LRBA_HUMAN	Lipopolysaccharide-responsive and beige-like anchor protein (CDC4-like protein) (Beige-like protein).	203 PDAF <u>FN</u> FPGKSAAAI
18 MM12_HUMAN	Macrophage metalloelastase precursor (EC 3.4.24.65) (HME) (Matrix metalloproteinase-12) (MMP-12) (Macrophage elastase) (ME).	367 SIHSF <u>GFPN</u> FVKKID
19 NDR4_HUMAN	NDRG4 protein (Brain development-related molecule 1) (Vascular smooth muscle cell associated protein-8) (SMAP-8).	80 FPQGYQF <u>PSME</u> QLAA
20 NOCT_HUMAN	Nocturnin (CCR4 protein homolog).	404 RLP <u>SFN</u> YPSDHL <sup>SLV</sup>
21 KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP- 1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	405 SFP <u>HYG</u> FPTYGGITF
22 KBF1_HUMAN	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP- 1) [Contains: Nuclear factor NF-kappa-B p50 subunit].	400 TGP <u>GYSF</u> PHYGFPTY
23 PAR2_HUMAN	Proteinase activated receptor 2 precursor (PAR-2) (Thrombin receptor- like 1) (Coagulation factor II receptor-like 1).	247 AIGV <u>FLF</u> PAFLTASA
24 LGR7_HUMAN	Relaxin receptor 1 (Leucine-rich repeat-containing G protein-coupled receptor 7).	734 KPD <u>LFTY</u> PCMSLIS
25 RRA_HUMAN	Retinoic acid receptor alpha (RAR-alpha).	22 PPYA <u>FFFFP</u> MLGGLS
26 ST5A_HUMAN	Signal transducer and activator of transcription 5A.	664 SYLI <u>VVFP</u> DRPKDEV
27 ST5B_HUMAN	Signal transducer and activator of transcription 5B.	664 NYLI <u>VVFP</u> DRPKDEV
28 SX12_HUMAN	SOX-12 protein (SOX-22 protein).	281 GTSH <u>FEFP</u> DYCTPEV
29 15277232	TNFalpha-inducible ATP-binding protein [Homo sapiens]	570 YTVR <u>FTFP</u> DPPLSP
30 TLR1_HUMAN	Toll-like receptor 1 precursor (Toll/interleukin-1 receptor-like) (TIL).	308 VSDV <u>F</u> GFPQSYIEI
31 TLR4_HUMAN	Toll-like receptor 4 precursor (hToll).	71 SYSF <u>SFPE</u> LQVLDL
32 GAT3_HUMAN	Trans-acting T-cell specific transcription factor GATA-3.	147 SPHL <u>FTFP</u> PTPKDV
33 TF65_MOUSE	Transcription factor p65 (Nuclear factor NF-kappa-B p65 subunit).	345 APQY <u>TFP</u> ASLSTIN
34 SX11_HUMAN	Transcription factor SOX-11.	407 LGS <u>HFEFP</u> DYCTPEL
35 SX21_HUMAN	Transcription factor SOX-21.	88 KKD <u>KFAFP</u> VPYGLGG
36 SOX4_HUMAN	Transcription factor SOX-4.	440 SGS <u>HFEFP</u> DYCTPEV
37 SOX6_HUMAN	Transcription factor SOX-6.	285 AQQG <u>FLFP</u> PGITYKP
38 SSRA_HUMAN	Translocon-associated protein, alpha subunit precursor (TRAP-alpha) (Signal sequence receptor alpha subunit) (SSR-alpha).	120 LDAS <u>FRYP</u> QDYQFYI
39 T11B_HUMAN	Tumor necrosis factor receptor superfamily member 11B precursor (Osteoprotegerin) (Osteoclastogenesis inhibitory factor).	351 HSKTY <u>HFPK</u> TVTQSL
40 T13X_HUMAN	Tumor necrosis factor receptor superfamily member 13B (Transmembrane activator and CAML interactor).	228 ETC <u>SFCF</u> PECRAPTQ
41 AG2R_HUMAN	Type-1 angiotensin II receptor (AT1) (AT1AR).	200 NILG <u>FLFP</u> FLILTS
42 JAK2_HUMAN	Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).	114 YRIR <u>FYF</u> PRWYCSGS
43 JAK3_HUMAN	Tyrosine-protein kinase JAK3 (EC 2.7.1.112) (Janus kinase 3) (JAK-3) (Leukocyte janus kinase) (L-JAK).	100 YRIR <u>FYF</u> PNWFGLEK
44 VNN3_HUMAN	Vascular non-inflammatory molecule 3 precursor (Vanin 3).	185 PEIQ <u>FDFP</u> KDSELVT
45 VNN3_HUMAN	Vascular non-inflammatory molecule 3 precursor (Vanin 3).	436 FGTR <u>YVFP</u> QIILSGS

565 RRTGFSFPTQEPRPQ  
352 GRRDFVYPSSTRDPS  
  
1255 KSEKFSWPQRSETLS

46 VINE\_HUMAN Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).  
47 VINE\_HUMAN Vinexin (SH3-containing adaptor molecule-1) (SCAM-1).  
Zinc finger protein 40 (Human immunodeficiency virus type I enhancer- binding protein 1) (HIV-EP1)  
(Major histocompatibility complex binding protein 1) (MBP-1) (Positive regulatory domain II binding  
factor 1) (PRDII-BF1).

48 ZEP1\_HUMAN

TABLE 5: METABOLIC DISORDERS

Accession Code	Target Description	Amino Acid	Target Sequence
1 PIP3_HUMAN	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta 3 (EC 3.1.4.11) (Phosphoinositide phospholipase C) (PLC-beta-3) (Phospholipase C-beta-3).	777 DEEPFDFPKVVLPTL	
2 AAK1_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-1 chain (EC 2.7.1.-) (AMPK alpha-1 chain).	273 DLPKYLFPEDPSSYSS	
3 AAK2_HUMAN	5'-AMP-activated protein kinase, catalytic alpha-2 chain (EC 2.7.1.-) (AMPK alpha-2 chain).	271 DLPSYLFPEDPSTYDA	
4 ACDV_HUMAN	Acyl-CoA dehydrogenase, very-long-chain specific, mitochondrial precursor (EC 1.3.99.-) (VLCAD).	84 TDQVFPYPVSVLNEEQ	
5 ANDR_HUMAN	Androgen receptor (Dihydrotestosterone receptor).	359 SRDYYNFPLALAGPP	
6 ANDR_HUMAN	Androgen receptor (Dihydrotestosterone receptor).	547 LPIDYYFPPQKTCLI	
7 APB_HUMAN	Apolipoprotein B-100 precursor (Apo B-100) [Contains: Apolipoprotein B-48 (Apo B-48)].	4198 NFPRFQFPKGPGIYT	
8 APL3_HUMAN	Apolipoprotein L3 (Apolipoprotein L-III) (ApoL-III) (TNF-inducible protein CG12-1) (CG12_1).	23 QVVTFTFPFGFQGIS	
9 AQP1_HUMAN	Aquaporin-CHIP (Water channel protein for red blood cells and kidney proximal tubule) (Aquaporin 1) (AQP-1) (Urine water channel). ATP-binding cassette, sub-family A, member 1 (ATP-binding cassette transporter 1) (ATP-binding cassette 1) (ABC-1) (Cholesterol efflux regulatory protein).	31 SALGFKYPVGNNQTA	
10 ABC1_HUMAN	ATP-binding cassette, sub-family F, member 2 (Iron inhibited ABC transporter 2) (HUSSY-18).	78 NNPCFRYPTPGEAPG	
11 ABF2_HUMAN	Beta-hexosaminidase alpha chain precursor (EC 3.2.1.52) (N-acetyl- beta-glucosaminidase) (Beta-N- acetylhexosaminidase) (Hexosaminidase A).	379 KTLSEYFPPCGKIPP	
12 HEXA_HUMAN	Bile salt export pump (ATP-binding cassette, sub-family B, member 11).	212 PYESFTFPELMRKGS	
13 AB11_HUMAN	Biliverdin reductase A precursor (EC 1.3.1.24) (Biliverdin-IX alpha- reductase).	1081 VDCKFTYPSRPDSQV	
14 BIEA_HUMAN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	158 EEERFGFPAFSGISR	
15 CEBA_BOVIN	CCAAT/enhancer binding protein alpha (C/EBP alpha).	27 SSAAFGFPRGAGPSQ	
16 CEBA_HUMAN	CCAAT/enhancer binding protein epsilon (C/EBP epsilon).	27 SSAAFGFPRGAGPAQ	
17 CEBE_HUMAN	channel-kinase 1 [Homo sapiens]	84 DPRPFAYPPTHFGPD	
18 13562153	Chloride channel protein 3 (CLC-3).	1339 QRKEFNFFEAGSSSG	
19 CLC3_HUMAN	Chloride channel protein 3 (CLC-3).	236 NIFSYLEPKYSTNEA	
20 CLC3_HUMAN	Chloride channel protein 6 (CLC-6).	280 EEVSYYFPLKTLWRS	
21 CLC6_HUMAN	Chloride channel protein precursor (Lipid transfer protein I).	223 RKIQFNFPYFRSDRD	
22 CICL_HUMAN		442 ETLSFIFPEGNVAGG	
23 CL16_HUMAN		638 KYRDFEFPSEMTGIW	
24 CETP_HUMAN		474 LQMDFGFPEHLLVDF	

25 CNG4_HUMAN	Cyclic-nucleotide-gated cation channel 4 (CNG channel 4) (CNG-4) (CNG4) (Cyclic nucleotide-gated cation channel modulatory subunit).	296 PWKKYQFPQSIDPLT
26 C561_HUMAN	Cytochrome b561 (Cytochrome b-561).	139 GFSFFLFPGASFSLR
27 CX42_HUMAN	Cytochrome c oxidase subunit IV isoform 2, mitochondrial precursor (EC 1.9.3.1) (COX IV-2).	122 WQRVVVFPPKPITLT
28 CP27_HUMAN	Cytochrome P450 27, mitochondrial precursor (EC 1.14.-.-) (Cytochrome P-450C27/25) (Sterol 26-hydroxylase) (Sterol 27-hydroxylase) (Vitamin D(3) 25-hydroxylase) (5-beta-cholestane-3-alpha,7-alpha,12-alpha-triol 27-hydroxylase).	412 EVDGFLFPKNTQFVF
29 12832024	dJ686C3.1.3 (isocitrate dehydrogenase 3 (NAD <sup>+</sup> ) beta (isoform C)) [Homo sapiens]	7 WSSLFPFPVSPSCCF
30 ENP4_HUMAN	Ectonucleoside triphosphate diphosphohydrolase 4 (EC 3.6.1.6) (NTPDase4) (Uridine-diphosphatase) (UDPase) (Lysosomal apyrase-like protein of 70 kDa).	500 FHRGFSFPVNYKSLK
31 EAA1_HUMAN	Excitatory amino acid transporter 1 (Sodium-dependent glutamate/aspartate transporter 1) (Glial glutamate transporter) (GLAST-1).	77 EVKYFSFPGELLMRM
32 EAA4_HUMAN	Excitatory amino acid transporter 4 (Sodium-dependent glutamate/aspartate transporter).	85 QIKYFSFPGELLMRM
33 EAA5_HUMAN	Excitatory amino acid transporter 5 (Retinal glutamate transporter).	46 EISYFQFPGELLMRM
34 F16P_HUMAN	Fructose-1,6-bisphosphatase (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase) (FBPase).	258 YGGIFLYPANKKSPN
35 F16Q_HUMAN	Fructose-1,6-bisphosphatase isozyme 2 (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase) (FBPase).	259 YGGIFLYPANQKSPK
36 CST_HUMAN	Galactosylceramide sulfotransferase (EC 2.8.2.11) (GalCer sulfotransferase) (Cerebroside sulfotransferase) (3'-phosphoadenylylsulfate:galactosylceramide 3'-sulfotransferase) (3'-phosphoadenosine-5'-phosphosulfate:GalCer sulfotransferase).	102 HRLKFAFPNGRNDFFD
37 KLK1_HUMAN	Glandular kallikrein 1 precursor (EC 3.4.21.35) (Tissue kallikrein) (Kidney/pancreas/salivary gland kallikrein).	162 EPENFSFPDDLQCVD
38 DCE2_HUMAN	Glutamate decarboxylase, 65 kDa isoform (EC 4.1.1.15) (GAD-65) (65 kDa glutamic acid decarboxylase).	131 KVIDFHYPNELLQEY
39 GSHR_HUMAN	Glutathione reductase, mitochondrial precursor (EC 1.8.1.7) (GR) (GRase).	125 DHADYGFPSCEGKFN
40 GCSP_HUMAN	Glycine dehydrogenase [decarboxylating], mitochondrial precursor (EC 1.4.4.2) (Glycine decarboxylase) (Glycine cleavage system P- protein).	260 SGVLFQYPDTEGKVE
41 GDE_HUMAN	Glycogen debranching enzyme (Glycogen debrancher) [Includes: 4-alpha-glucanotransferase (EC 2.4.1.25) (Oligo-1,4-1,4-glucantransferase); Amylo-alpha-1,6-glucosidase (EC 3.2.1.33) (Amylo-1,6-glucosidase) (Dextrin 6-alpha-D-glucosidase)].	426 VTRYFTFPFEEIDFS
42 KG3A_HUMAN	Glycogen synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	350 NYTEFKFPQIKAHPW
43 SYG_HUMAN	Glycyl-tRNA synthetase (EC 6.1.1.14) (Glycine--tRNA ligase) (GlyRS).	601 QRTFFSFPVAVAPFK
44 GUAD_HUMAN	Guanine deaminase (EC 3.5.4.3) (Guanase) (Guanine aminase) (Guanine aminohydrolase) (GAH) (p51-nedasin).	102 WLTKYTFPAEHRFQN
45 HO1_HUMAN	Heme oxygenase 1 (EC 1.14.99.3) (HO-1).	163 GLAFFITFPNIASATK
46 HNFB_HUMAN	Hepatocyte nuclear factor 1-beta (HNF-1B) (Variant hepatic nuclear factor 1) (VHNF1)	200 DQLLFLFPEFSQQSH
47 HXK4_HUMAN	(Homeoprotein LFB3) (Transcription factor 2) (TCF-2).	146 LGFTFSFPVRHEDID
	Hexokinase D (EC 2.7.1.1) (Hexokinase type IV) (HK IV) (HK4) (Glucokinase).	

163 LGFSFSFPCHQTGLD  
598 LGFTFSFPCQQTSLD  
598 LGFTFSFPCQQNSLD  
599 FVRFYPYPCQTTELA  
280 QFHSEIYPYMANGL  
78 VSAF~~G~~FPVILARVA  
142 MEEEFNYPLDNDVHLL

1025 QCGLFSFPCCKNGRCV  
173 YPQQFPYPQAPFVSQ  
281 DDL~~L~~YSFPPV~~I~~KNKT  
227 VRNTYIFPPEPSMKI

113 KNTSFAYPAIRYLLY  
2 HGQTFTFPDLFPEKD  
30 IGFSYAFPKSITVFF  
30 IGFSYAFPKAVTVFF  
29 TGFAYGFPKAVSVFF  
32 TGFSYAFPKAVSVFF  
306 STNPFDFFPVSQGEV

37 MVSDFFYPNMGGVES  
24 FPPDYGFPERKEREM

75 HPQPYIFPDSPGGTS  
452 GNNSYVFP~~G~~VALGW  
669 TFQFYRFPPATTPRL  
1295 NAQQFPPFPN~~Y~~GISQ  
23 HSVQYTFPNTRHQQE

2160 PAPLYSFPGASCPVL  
540 PPSPFSEFPMNPGGWS  
365 QLLVFMFPVGLYYCF  
513 CLNEFNFPDPYSKVK  
417 PDDIFLFPKLLQKMA  
389 PDSQYLF~~P~~KLLQKMA

48 HXK3\_HUMAN

49 HXK1\_HUMAN

50 HXK2\_HUMAN

51 IKAP\_HUMAN

52 IRA2\_HUMAN

53 OBRG\_HUMAN

54 LIPL\_HUMAN

55 LRP2\_HUMAN

56 LOL1\_HUMAN

57 MDHC\_HUMAN

58 MUTA\_HUMAN

59 RT29\_HUMAN

60 14141575

61 MOT1\_HUMAN

62 MOT2\_HUMAN

63 MOT3\_HUMAN

64 MOT4\_HUMAN

65 MYHD\_HUMAN

66 PIGA\_HUMAN

67 NB8M\_HUMAN

68 NI2M\_HUMAN

69 MAOX\_HUMAN

70 NPH4\_HUMAN

71 NCO2\_HUMAN

72 NCR1\_HUMAN

73 NCR2\_HUMAN

74 RORG\_HUMAN

75 STT3\_HUMAN

76 PNK4\_HUMAN

77 PPAR\_HUMAN

78 PPAS\_MOUSE

Hexokinase type III (EC 2.7.1.1) (HK III).

Hexokinase, type I (EC 2.7.1.1) (HK I) (Brain form hexokinase).

Hexokinase, type II (EC 2.7.1.1) (HK II) (Muscle form hexokinase).

IkappaB kinase complex-associated protein (IKK complex-associated protein) (p150).

Interleukin-1 receptor-associated kinase-2 (EC 2.7.1.-) (IRAK-2).

Leptin receptor gene-related protein (OB-R gene related protein) (OB- RGRP).

Lipoprotein lipase precursor (EC 3.1.1.34) (LPL).

Low-density lipoprotein receptor-related protein 2 precursor (Megalin) (Glycoprotein 330) (gp330).

Lysyl oxidase homolog 1 precursor (EC 1.4.3.-) (Lysyl oxidase-like protein 1) (LOL).

Malate dehydrogenase, cytoplasmic (EC 1.1.1.37).

Methylmalonyl-CoA mutase, mitochondrial precursor (EC 5.4.99.2) (MCM).

Mitochondrial 28S ribosomal protein S29 (S29mt) (MRP-S29) (Death- associated protein 3) (DAP-3) (Ionizing radiation resistance conferring protein).

mitochondrial ribosomal protein L23 [Homo sapiens]

Monocarboxylate transporter 1 (MCT 1).

Monocarboxylate transporter 2 (MCT 2).

Monocarboxylate transporter 3 (MCT 3).

Monocarboxylate transporter 4 (MCT 4) (MCT 3).

Myosin heavy chain, skeletal muscle, extraocular (MyHC-eo).

N-acetylglucosaminyl-phosphatidylinositol biosynthetic protein (GlcNAc-PI synthesis protein)

(Phosphatidylinositol-glycan biosynthesis, class A protein) (PIG-A).

NADH-ubiquinone oxidoreductase B18 subunit (EC 1.6.5.3) (EC 1.6.99.3) (Complex I-B18) (CI-B18)

(Cell adhesion protein SQM1).

NADH-ubiquinone oxidoreductase B22 subunit (EC 1.6.5.3) (EC 1.6.99.3) (Complex I-B22) (CI-B22).

NADP-dependent malic enzyme (EC 1.1.1.40) (NADP-ME) (Malic enzyme 1).

Nephrocystin 4 (Nephroretinin).

Nuclear receptor coactivator 2 (NCoA-2) (Transcriptional intermediary factor 2).

Nuclear receptor co-repressor 1 (N-CoR1) (N-CoR).

Nuclear receptor co-repressor 2 (N-CoR2) (Silencing mediator of retinoic acid and thyroid hormone receptor) (SMRT) (SMRTE) (Thyroid-, retinoic-acid-receptor-associated co-repressor) (T3 receptor- associating factor) (TRAC) (CTG repeat protein 26).

Nuclear receptor ROR-gamma (Nuclear receptor RZR-gamma).

Oligosaccharyl transferase STT3 subunit homolog (B5) (Integral membrane protein 1) (TMC).

Pantothenate kinase 4 (EC 2.7.1.33) (Pantothenic acid kinase 4) (hPank4).

Peroxisome proliferator activated receptor alpha (PPAR-alpha).

Peroxisome proliferator activated receptor delta (PPAR-delta) (PPAR-beta) (Nuclear hormone

receptor 1) (NUC1).

79	P11D_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, delta isoform (EC 2.7.1.153) (PI3-kinase p110 subunit delta) (PtdIns- 3-kinase p110) (PI3K) (p110delta).	581	ELLDFSFPDCHVGSF
80	PIGO_HUMAN	Phosphatidylinositol-glycan biosynthesis, class O protein (PIG-O).	190	FSKAFFFFSFNVRDL
81	940231	phosphodiesterase A' subunit [Homo sapiens]	366	ADEYFTFPKGPVDET
82	KPRA_HUMAN	Phosphoribosyl pyrophosphate synthetase-associated protein 1 (PRPP synthetase-associated protein 1) (39 kDa phosphoribosyrophosphate synthetase-associated protein) (PAP39).	140	IQGFFSFVDNLRAS
83	PIT1_HUMAN	Pituitary-specific positive transcription factor 1 (Pit-1) (Growth hormone factor 1) (GHF-1).	75	TPCLYKFPDHTLSHG
84	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Tigmin).	3321	DKNKFYFPSLQPRKD
85	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Tigmin).	2512	NLVAFPFPHAAILED
86	PKHD_HUMAN	Polycystic kidney and hepatic disease 1 precursor (Fibrocystin) (Polyductin) (Tigmin).	2370	RYGLFVYPKFQPPWD
87	P1L1_HUMAN	Polycystic kidney disease 1-like 1 protein (Polycystin 1L1).	2687	PGLLFHFPRRSQKDC
88	PKDR_HUMAN	Polycystic kidney disease and receptor for egg jelly related protein precursor (PKD and REJ homolog).	1880	GYALYFFPEQQRFNS
89	PKD1_HUMAN	Polycystin 1 precursor (Autosomal dominant polycystic kidney disease protein 1).	3060	SHVRFVFPEPTADVN
90	PAGT_HUMAN	Polypeptide N-acetylgalactosaminyltransferase (EC 2.4.1.41) (Protein- UDP	348	KATPYTFPGGTGQII
91	DPI_HUMAN	acetylgalactosaminyltransferase) (UDP-GalNAc:polypeptide, N- acetylgalactosaminyltransferase) (GalNAc-T1).	56	NLIGFGYPAYISIKA
92	CIK1_HUMAN	Polyposis locus protein 1 (TB2 protein).	153	VWLLFEYPESSGPAP
93	CIK5_HUMAN	Potassium voltage-gated channel subfamily A member 1 (Potassium channel Kv1.1) (HUK1) (HBK1).	236	VWLIFEYPESSGSAR
94	KCH7_HUMAN	Potassium voltage-gated channel subfamily H member 7 (Ether-a-go-go related gene potassium channel 3) (HERG-3) (Ether-a-go-go related protein 3) (Eag related protein 3).	156	KFFGFKFPGLRVLT
95	S24C_HUMAN	Protein transport protein Sec24C (SEC24-related protein C).	942	ETNVFFYPRLPLTK
96	6007826	rab escort protein-2 [Mus musculus]	370	GNTPFIFPLYGHGEI
97	NPT2_HUMAN	Renal sodium-dependent phosphate transport protein 2 (Sodium/phosphate cotransporter 2)	43	GTSAYAFPSLGPVAL
98	RRA_HUMAN	(Na(+)/Pi cotransporter 2) (Renal sodium-phosphate transport protein 2) (Renal Na(+)-dependent phosphate cotransporter 2).	22	PPYAFFFFPPMLGGLS
99	RRG1_HUMAN	Retinoic acid receptor alpha (RAR-alpha).	23	AGFPFAFPALRGSP
100	RRG1_HUMAN	Retinoic acid receptor gamma-1 (RAR-gamma-1).	370	PSQPYMFPRMLMKIT
101	12053793	Retinoic acid receptor gamma-1 (RAR-gamma-1).	1830	PISLFSFPPLLPQQF
102	SIL5_HUMAN	retinoid-acid induced protein 1 [Homo sapiens]	39	VPCSFSYPWRSWYSS
103	S6A9_HUMAN	Sialic acid binding Ig-like lectin 5 precursor (Siglec-5) (Obesity- binding protein 2) (OB binding protein-2) (OB-BP2) (CD33 antigen-like 2) (CD170 antigen).	120	GGGAFMFYFIMLIF
104	NTCP_HUMAN	Sodium- and chloride-dependent glycine transporter 1 (GlyT1) (GlyT-1).	279	IGPLFFFFLLYMIFQ
		Sodium/bile acid cotransporter (Na(+)/bile acid cotransporter) (Na(+)/taurocholate transport protein) (Sodium/taurocholate cotransporting polypeptide).		

105 NAH5_HUMAN	Sodium/hydrogen exchanger 5 (Na(+)/H(+) exchanger 5) (NHE-5).	835 PRSSFAFP <sup>PS</sup> SLAKAG
106 S6A7_HUMAN	Sodium-dependent proline transporter.	525 EYGSYRFP <sup>PP</sup> WAE <sup>LL</sup> LG
107 S21B_HUMAN	Solute carrier family 21 member 11 (Sodium-independent organic anion transporter D) (Organic anion transporting polypeptide D) (OATP-D) (Organic anion transporter polypeptide related protein 3) (OATP-RP3) (OATPRP3) (PGE1 transporter).	279 SLLMFGFP <sup>QS</sup> SLP <sup>PH</sup> S
108 SYTA_MOUSE	Synaptotagmin X (SytX).	296 FDELFQFP <sup>VV</sup> YD <sup>QL</sup> S
109 SYTB_HUMAN	Synaptotagmin XI (SytXI).	163 FSVDYNFP <sup>KK</sup> AL <sup>V</sup> VT
110 T240_HUMAN	Thyroid hormone receptor-associated protein complex 240 kDa component (Trap240) (Activator-recruited cofactor 250 kDa component) (ARC250).	703 GDEEFLFP <sup>DK</sup> KK <sup>DR</sup> QN
111 T240_HUMAN	Thyroid hormone receptor-associated protein complex 240 kDa component (Trap240) (Activator-recruited cofactor 250 kDa component) (ARC250).	614 AWKYYKFP <sup>KK</sup> KD <sup>VE</sup> F
112 TRIB_HUMAN	Thyroid receptor interacting protein 12 (TRIP12).	1536 KTCPPFFFP <sup>FD</sup> TR <sup>QM</sup> L
113 15277232	TNFalpha-inducible ATP-binding protein [Homo sapiens]	570 YTVRFIFP <sup>DP</sup> PP <sup>PL</sup> SP
114 TERA_HUMAN	Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)-ATPase p97 subunit) (Valosin containing protein) (VCP) [Contains: Valosin].	767 GFGSFRFP <sup>SG</sup> NQ <sup>GG</sup> A
115 TUL2_HUMAN	Tubby related protein 2 (Tubby-like protein 2).	498 FTMDFCFP <sup>FS</sup> PL <sup>QA</sup> F
116 TUSP_HUMAN	Tubby superfamily protein.	1518 YILDFQYP <sup>FS</sup> AV <sup>QA</sup> F
117 T11B_HUMAN	Tumor necrosis factor receptor superfamily member 11B precursor (Osteoprotegerin) (Osteoclastogenesis inhibitory factor).	351 HSKTYHFP <sup>KT</sup> VT <sup>QS</sup> L
118 T13X_HUMAN	Tumor necrosis factor receptor superfamily member 13B (Transmembrane activator and CAML interactor).	228 ETCSECFP <sup>EC</sup> RAP <sup>TQ</sup>
119 JAK2_HUMAN	Tyrosine-protein kinase JAK2 (EC 2.7.1.112) (Janus kinase 2) (JAK-2).	114 YRIRFYFP <sup>RW</sup> YC <sup>SG</sup> S
120 UDBF_HUMAN	UDP-glucuronosyltransferase 2B15 precursor, microsomal (EC 2.4.1.17) (UDPGT) (UDPGTH-3) (HLUG4).	184 NGGGFLFP <sup>PS</sup> YV <sup>PV</sup> W
121 V1BR_HUMAN	Vasopressin V1b receptor (V1bR) (AVPR V1b) (Vasopressin V3 receptor) (AVPR V3) (Antidiuretic hormone receptor 1b).	186 CWADFGFP <sup>W</sup> GPR <sup>AY</sup> L
122 16904210	very large G protein-coupled receptor 1 [Mus musculus]	1971 PYGVFI <sup>FP</sup> NK <sup>TR</sup> PL <sup>S</sup>
123 WRN_HUMAN	Werner syndrome helicase.	577 KSLCFQYP <sup>PP</sup> VV <sup>VG</sup> KI
124 WFS1_HUMAN	Wolfraamin.	413 FFVIFSFP <sup>PI</sup> ASK <sup>DC</sup> I
125 ZN44_HUMAN	Zinc finger protein 44 (Zinc finger protein KOX7) (Gonadotropin inducible transcription repressor-2) (GIOT-2).	342 CGKGFD <sup>FP</sup> GSAR <sup>I</sup> HE

TABLE 6: NEUROPATHIES

Accession Code	Target Description	Amino Acid	Target Sequence
1 5H1B_HUMAN	5-hydroxytryptamine 1B receptor (5-HT-1B) (Serotonin receptor) (5-HT-1D-beta) (Serotonin 1D beta receptor) (S12).	213	TVGAFY <u>F</u> P <u>T</u> LLIAL
2 5H2C_HUMAN	5-hydroxytryptamine 2C receptor (5-HT-2C) (Serotonin receptor) (5HT-1C).	42	DGGRFKFPDGVQNWPP

3	5HT3_HUMAN	5-hydroxytryptamine 3 receptor precursor (5-HT-3) (Serotonin-gated ion channel receptor) (5-HT3R).	158	<u>SLDIYNFPFDVQNCS</u>
4	AUT2_HUMAN	Autism susceptibility gene 2 protein.	1057	<u>GGERFPYPSFHWDP</u>
5	BIR1_HUMAN	Baculoviral IAP repeat-containing protein 1 (Neuronal apoptosis inhibitory protein).	649	<u>CAHWFEQYPFDPSFDD</u>
6	BRA1_HUMAN	Brain link protein-1 precursor.	318	<u>GVRSEFGFPRPQQAAY</u>
7	BAI1_HUMAN	Brain-specific angiogenesis inhibitor 1 precursor.	341	<u>ELQQFGFPAPQTGDP</u>
8	CBL2_HUMAN	Cdk5 and abl enzyme substrate 2 (Interactor with cdk3 2) (Ik3-2).	241	<u>SYAKFLYPTNALVTH</u>
9	DYHB_HUMAN	Ciliary dynein heavy chain 11 (Axonemal beta dynein heavy chain 11).	2649	<u>TVFAFNFP<del>SLDALNT</del></u>
10	FMO5_HUMAN	Dimethylalanine monooxygenase [N-oxide forming] 5 (EC 1.14.13.8) (Hepatic flavin-containing monooxygenase 5) (FMO 5) (Dimethylalanine oxidase 5).	329	<u>TGYSFD<del>FPFLED</del>SVK</u>
11	DIS1_HUMAN	Disrupted in schizophrenia 1 protein.	61	<u>VGTLFRFP<del>GGVSGEE</del></u>
12	DOPO_MOUSE	Dopamine beta-monooxygenase precursor (EC 1.14.17.1) (Dopamine beta- hydroxylase) (DBH).	309	<u>GAKAFYYPKEAGVPF</u>
13	DSCA_HUMAN	Down syndrome cell adhesion molecule precursor (CHD2).	80	<u>TLQIFPFP<del>PPSS</del>FSTL</u>
14	DSCA_HUMAN	Down syndrome cell adhesion molecule precursor (CHD2).	598	<u>FIQPFEEFPRFSIGQR</u>
15	DSR3_HUMAN	Down syndrome critical region protein 3 (Down syndrome critical region protein A).	89	<u>TEIPFEEFPLHLKGNK</u>
16	DYSF_HUMAN	Dysferlin (Dystrophy associated fer-1-like protein) (Fer-1 like protein 1).	1867	<u>FNWRFIFPFDYLP<del>PAE</del></u>
17	EPA7_HUMAN	Ephrin type-A receptor 7 precursor (EC 2.7.1.112) (Tyrosine-protein kinase receptor EHK-3) (Eph homology kinase-3) (Receptor protein- tyrosine kinase HEK11).	596	<u>LYFHF<del>FKFP</del>GTKTYID</u>
18	ERC6_HUMAN	Excision repair protein ERCC-6 (Cockayne syndrome protein CSB).	687	<u>SLFD<del>FIFP</del>GKLGTL<del>P</del></u>
19	EAA3_HUMAN	Excitatory amino acid transporter 3 (Sodium-dependent glutamate/aspartate transporter 3)	48	<u>EKFYFAFPGEILMRM</u>
20	FXGA_HUMAN	(Excitatory amino-acid carrier 1) (Neuronal and epithelial glutamate transporter).	391	<u>GQTSYFFPHVPHPSM</u>
21	FMR2_HUMAN	Forkhead box protein G1A (Forkhead-related protein FKHL2) (Transcription factor BF-2) (Brain factor 2) (BF2) (HFK2).	639	<u>STDEFTWPKNITSS</u>
22	FMR2_HUMAN	Fragile X mental retardation 2 protein (Protein FMR-2) (FMR2P) (Ox19 protein) (Fragile X E mental retardation syndrome protein).	193	<u>LEDDFFVPAEQPQIG</u>
23	FCMD_HUMAN	Fragile X mental retardation 2 protein (Protein FMR-2) (FMR2P) (Ox19 protein) (Fragile X E mental retardation syndrome protein).	388	<u>KKFKYLFPKFTLCWT</u>
24	GAE_HUMAN	Fukutin precursor (Fukuyama-type congenital muscular dystrophy protein).	212	<u>SFSSFSYPENEM<del>YK</del></u>
25	GAAT_HUMAN	Gamma-aminobutyric-acid receptor epsilon subunit precursor (GABA(A) receptor).	609	<u>KWSRFLFPLAFGLFN</u>
26	SGCG_HUMAN	Gamma-aminobutyric-acid receptor theta subunit precursor (GABA(A) receptor).	81	<u>GESEFLFPLYAKEIH</u>
27	KG3A_HUMAN	Gamma-sarcoglycan (Gamma-SG) (35 kDa dystrophin-associated glycoprotein) (35DAG).	350	<u>NYTEFKFPQIK<del>AHPW</del></u>
28	17384611	Glycogen synthase kinase-3 alpha (EC 2.7.1.37) (GSK-3 alpha).	945	<u>DASSFFFPPISSCPP</u>
29	OPRK_HUMAN	kainate receptor subunit KA2a [Homo sapiens]	340	<u>CFRDFCFLKMRMER</u>
30	13383464	Kappa-type opioid receptor (KOR-1).	46	<u>NTRDFMFPGPNQMSG</u>
31	MGR1_HUMAN	kinesin-related protein HASH [Mus musculus]	3	<u>GLLLFFFFPAIFLEVS</u>
32	9988950	Metabotropic glutamate receptor 1 precursor (mGluR1).	9	<u>RYPDFSFYPFPQDYF</u>
33	MY15_HUMAN	monooxygenase X [Homo sapiens]	751	<u>RGAAFGFP<del>GASPRAS</del></u>
		Myosin XV (Unconventional myosin-15).		

34	MTR2_HUMAN	Myotubularin-related protein 2.	178	NLMKYAF <u>VPVSN</u> NLPL
35	2736151	mytonic dystrophy kinase-related Cdc42-binding kinase [Rattus norvegicus]	297	HKERFQFPTQVTDVS
36	NCA2_HUMAN	Neural cell adhesion molecule 1, 120 kDa isoform precursor (N-CAM 120) (NCAM-120) (CD56 antigen).	425	TCEVFAYPSATISWF
37	NTC3_HUMAN	Neurogenic locus notch homolog protein 3 precursor (Notch 3).	1618	ERLDFPYPLRDVRGE
38	NGN1_HUMAN	Neurogenin 1 (Neurogenic differentiation factor 3) (NeuroD3) (Neurogenic basic-helix-loop-helix protein).	213	GDPVFSFSPSLPKDLL
39	NTR1_HUMAN	Neurotensin receptor type 1 (NT-R-1) (High-affinity levocabastine- insensitive neurotensin receptor) (NTRH).	241	TFMSFIFPMVVISVL
40	AAAT_HUMAN	Neutral amino acid transporter B(0) (ATB(0)) (Sodium-dependent neutral amino acid transporter type 2) (RD114/simian type D retrovirus receptor) (Baboon M7 virus receptor).	85	RLSAFVFPGE <sup>1</sup> LLRL
41	OAA3_HUMAN	Olfactory receptor 10A3 (HTPCR12).	160	TTWVFSFPFCGPNEI
42	OAA5_HUMAN	Olfactory receptor 10A5 (HP3) (Olfactory receptor-like protein JCG6).	161	TTWLFSFPFCGTNKV
43	OXB2_HUMAN	Olfactory receptor 51B2 (HOR5'beta3).	275	SYIYFLFPPLMNPVI
44	OXB4_HUMAN	Olfactory receptor 51B4 (HOR5'beta1).	274	SYVHFLFPPFVNP <sup>1</sup> II
45	OXI1_HUMAN	Olfactory receptor 51I1 (HOR5'beta11).	155	FTTLFPPFPFVKRLP
46	O6B1_HUMAN	Olfactory receptor 6B1 (Olfactory receptor 7-3) (OR7-3).	203	ALVIFLFLFITVLS
47	PAN1_HUMAN	Pannexin 1.	187	SESHFKYPIVEQYLK
48	RELN_HUMAN	Reelin precursor (EC 3.4.21.-).	1894	LMDEFYFPQT <sup>1</sup> TNILF
49	RELN_HUMAN	Reelin precursor (EC 3.4.21.-).	1967	EDNWFFYPGGNIGLY
50	SACS_HUMAN	Sacsin.	1944	NCTMFRFPLRNAEMA
51	SAD1_HUMAN	SAM domain and HD domain-containing protein 1 (Dendritic cell-derived IFNG-induced protein) (DCIP) (Monocyte protein 5) (MOP-5).	151	GGGYVVFPGASHNRF
52	KPT3_HUMAN	Serine/threonine protein kinase PCTAIRE-3 (EC 2.7.1.-).	371	EFRTYSFPCYLPQPL
53	SAMP_HUMAN	Serum amyloid P-component precursor (SAP) (9.5S alpha-1-glycoprotein).	24	SGKVVFPPRESVTDH
54	SHK2_RAT	SH3 and multiple ankyrin repeat domains protein 2 (Shank2) (Proline- rich synapse associated protein 1) (ProSAP1) (Cortactin-binding protein 1) (CortBP1) (GKAP/SAPAP interacting protein) (SPANK-3).	131	SLSTFEYPGPRRKLY
55	20073220	similar to dJ309K20.4 (KIAA0765, putative brain nuclearly targeted protein (HRIHFB2091, RNA recognition motif (RNP, RRM or RBD domain) containing protein)) [Mus musculus]	631	NGPPFNFPNGFGGPN
56	20071167	Similar to Per1 interacting protein [Mus musculus]	551	PMNPF <sup>1</sup> RFPPKEAASLF
57	S6A1_HUMAN	Sodium- and chloride-dependent GABA transporter 1.	523	TMGNYVFPPK <sup>1</sup> WGQGVG
58	SX14_HUMAN	Transcription factor SOX-14.	88	KKDRYVFPLPYLGDT
59	ROR1_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 1).	785	RYPNYMFPSQGITPQ
60	ROR1_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR1 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 1).	226	SLCHYAFPYCDETSS
61	ROR2_HUMAN	Tyrosine-protein kinase transmembrane receptor ROR2 precursor (EC 2.7.1.112) (Neurotrophic tyrosine kinase, receptor-related 2).	230	SFCHFVFLCDARSR

62 WS14\_HUMAN

63 WFS1\_HUMAN

Williams-Beuren syndrome chromosome region 14 protein (WS basic-helix- loop-helix leucine zipper protein) (WS-bHLH) (Mlx interactor).  
Wolframin.

431 FSPRFPFPTVPPAPG

413 FFVIFSFPIASKDCI

TABLE 7: MISCELLANEOUS DEF DOMAIN-CONTAINING PROTEINS

Accession Code	Target Description	Amino Acid	Target Sequence
1 15425674	Per1 interacting protein of the suprachiasmatic nucleus [Rattus norvegicus]	1023	SLNP <u>F</u> R <u>F</u> P <u>K</u> EAA <u>S</u> LF
2 PER1_HUMAN	Period circadian protein 1 (Circadian pacemaker protein Rigui) (hPER).	922	VLPNYL <u>F</u> P <u>T</u> PS <u>S</u> YPY
3 PER2_HUMAN	Period circadian protein 2.	907	MLPSYS <u>F</u> P <u>S</u> GT <u>P</u> NLP
4 PER3_HUMAN	Period circadian protein 3 (hPER3). A-kinase anchor protein 11 (Protein kinase A anchoring protein 11) (PRKA11) (A kinase anchor protein 220 kDa) (AKAP 220) (hAKAP220).	843	PYP <u>A</u> <u>F</u> P <u>F</u> P <u>Y</u> LD <u>T</u> FMT
5 AK11_HUMAN	A-kinase anchor protein 3 (Protein kinase A anchoring protein 3) (PRKA3) (A-kinase anchor protein 110 kDa) (AKAP 110) (Sperm oocyte binding protein) (Fibrous sheath protein of 95 kDa) (FSP95).	661	EVCQ <u>F</u> SYPQTP <u>A</u> SPQ
6 AKA3_HUMAN		490	SDIS <u>F</u> EY <u>P</u> EDIGNLS